THE HARVEY LECTURES

THE HARVEY LECTURES

DELIVERED UNDER THE AUSPICES OF

The HARVEY SOCIETY of NEW YORK

1946-1947

———

UNDER THE PATRONAGE OF THE NEW YORK
ACADEMY OF MEDICINE

———

BY

DR. RAFAEL LORENTE DE NÓ   DR. ISAAC STARR
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DR. WILLIAM H. PETERSON
DR. SAM Z. LEVINE
DR. EUGENE F. DU BOIS

SERIES XLII

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CONTENTS

THE HARVEY SOCIETY

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE
OF THE MEDICAL SCIENCES

CONSTITUTION

I

This Society shall be named the Harvey Society.

II

The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology, and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented.

III

The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences, residing in the City of New York, who have personally contributed to the advancement of these sciences. Associate members shall be meritorious physicans who are in sympathy with the objects of the Society, residing in the City of New York. Members who leave New York to reside elsewhere may retain their membership. Honorary members shall be those who have delivered lectures before the Society and who are neither Active nor Associate members. Associate and Honorary members shall not be eligible to office, nor shall they be entitled to a vote.

Members shall be elected by ballot. They shall be nominated to the Executive Committee and the names of the nominees shall accompany the notice of the meeting at which the vote for their election will be taken.

IV

The management of the Society shall be vested in an Executive Committee to consist of a President, a Vice-President, a Secretary, a Treasurer, and three other members, these officers to be elected by ballot at each annual meeting of the Society to serve one year.

V

The Annual Meeting of the Society shall be held at a stated date in January of each year at a time and place to be determined by the Executive Committee. Special meetings may be held at such times and places as the Executive Committee may determine. At all meetings ten members shall constitute a quorum.

VI

Changes in the Constitution may be made at any meeting of the Society by a majority vote of those present after previous notification to the members in writing.

FORMER OFFICERS OF THE HARVEY SOCIETY

1905–1906

President: GRAHAM LUSK
Vice-President: SIMON FLEXNER
Treasurer: FREDERIC S. LEE
Secretary: GEORGE B. WALLACE

Council:
C. A. HERTER
S. J. MELTZER
EDWARD K. DUNHAM

1906–1907

President: GRAHAM LUSK
Vice-President: SIMON FLEXNER
Treasurer: FREDERIC S. LEE
Secretary: GEORGE B. WALLACE

Council:
C. A. HERTER
S. J. MELTZER
JAMES EWING

1907–1908

President: GRAHAM LUSK
Vice-President: JAMES EWING
Treasurer: EDWARD K. DUNHAM
Secretary: GEORGE B. WALLACE

Council:
SIMON FLEXNER
THEO. C. JANEWAY
PHILIP H. HISS, JR.

1908–1909

President: JAMES EWING
Vice-President: SIMON FLEXNER
Treasurer: EDWARD K. DUNHAM
Secretary: FRANCIS C. WOOD

Council:
GRAHAM LUSK
S. J. MELTZER
ADOLF MEYER

1909–1910*

President: JAMES EWING
Vice-President: THEO. C. JANEWAY
Treasurer: EDWARD K. DUNHAM
Secretary: FRANCIS C. WOOD

Council:
GRAHAM LUSK
S. J. MELTZER
W. J. GIES

* At the Annual Meeting of May 18, 1909, these officers were elected. In publishing the 1909–1910 volume their names were omitted, possibly because in that volume the custom of publishing the names of the incumbents of the current year was changed to publishing the names of the officers selected for the ensuing year.

1910–1911

President: SIMON FLEXNER   *Council:*
Vice-President: JOHN HOWLAND   GRAHAM LUSK
Treasurer: EDWARD K. DUNHAM   S. J. MELTZER
Secretary: HAVEN EMERSON   JAMES EWING

1911–1912

President: S. J. MELTZER   *Council:*
Vice-President: FREDERIC S. LEE   GRAHAM LUSK
Treasurer: EDWARD K. DUNHAM   JAMES EWING
Secretary: HAVEN EMERSON   SIMON FLEXNER

1912–1913

President: FREDERIC S. LEE   *Council:*
Vice-President: WM. H. PARK   GRAHAM LUSK
Treasurer: EDWARD K. DUNHAM   S. J. MELTZER
Secretary: HAVEN EMERSON   WM. G. MACCALLUM

1913–1914

President: FREDERIC S. LEE   *Council:*
Vice-President: WM. G. MACCALLUM   GRAHAM LUSK
Treasurer: EDWARD K. DUNHAM   WM. H. PARK
Secretary: AUGUSTUS B. WADSWORTH   GEORGE B. WALLACE

1914–1915

President: WM. G. MACCALLUM   *Council:*
Vice-President: RUFUS I. COLE   GRAHAM LUSK
Treasurer: EDWARD K. DUNHAM   FREDERIC S. LEE
Secretary: JOHN A. MANDEL   W. T. LONGCOPE

1915–1916

President: GEORGE B. WALLACE[*]   *Council:*
Treasurer: EDWARD K. DUNHAM   GRAHAM LUSK
Secretary: ROBERT A. LAMBERT   RUFUS I. COLE
    NELLIS B. FOSTER

[*] Dr. William G. MacCallum resigned after election. On Doctor Lusk's motion Doctor George B. Wallace was made President—no Vice-President was appointed.

1916–1917

President: George B. Wallace
Vice-President: Rufus I. Cole
Treasurer: Edward K. Dunham
Secretary: Robert A. Lambert

Council:
Graham Lusk*
W. T. Longcope
S. R. Benedict
Hans Zinsser

1917–1918

President: Edward K. Dunham
Vice-President: Rufus I. Cole
Treasurer: F. H. Pike
Secretary: A. M. Pappenheimer

Council:
Graham Lusk
George B. Wallace
Frederic S. Lee
Peyton Rous

1918–1919

President: Graham Lusk
Vice-President: Rufus I. Cole
Treasurer: F. H. Pike
Secretary: K. M. Vogel

Council:
Graham Lusk
James W. Jobling
Frederic S. Lee
John Auer

1919–1920

President: Warfield T. Longcope
Vice-President: S. R. Benedict
Treasurer: F. H. Pike
Secretary: K. M. Vogel

Council:
Graham Lusk
Hans Zinsser
Frederic S. Lee
George B. Wallace

1920–1921†

President: Warfield T. Longcope
Vice-President: S. R. Benedict
Treasurer: A. M. Pappenheimer
Secretary: Homer F. Swift

Council:
Graham Lusk
Frederic S. Lee
Hans Zinsser
George B. Wallace

* Doctor Lusk was made Honorary permanent Counsellor.

† These officers were elected at the Annual Meeting of May 21, 1920, but were omitted in the publication of the 1919–1920 volume.

1921–1922

President: RUFUS I. COLE
Vice-President: STANLEY R. BENEDICT
Treasurer: A. M. PAPPENHEIMER
Secretary: HOMER F. SWIFT

Council:
GRAHAM LUSK
HANS ZINSSER
H. C. JACKSON
W. T. LONGCOPE

1922–1923

President: RUFUS I. COLE
Vice-President: HANS ZINSSER
Treasurer: CHARLES C. LIEB
Secretary: HOMER F. SWIFT

Council:
GRAHAM LUSK
W. T. LONGCOPE
H. C. JACKSON
S. R. BENEDICT

1923–1924

President: EUGENE F. DUBOIS
Vice-President: HOMER F. SWIFT
Treasurer: CHARLES C. LIEB
Secretary: GEORGE M. MACKENZIE

Council:
GRAHAM LUSK
ALPHONSE R. DOCHEZ
DAVID MARINE
PEYTON ROUS

1924–1925

President: EUGENE F. DUBOIS
Vice-President: PEYTON ROUS
Treasurer: CHARLES C. LIEB
Secretary: GEORGE M. MACKENZIE

Council:
GRAHAM LUSK
RUFUS I. COLE
HAVEN EMERSON
WM. H. PARK

1925–1926

President: HOMER F. SWIFT
Vice-President: H. B. WILLIAMS
Treasurer: HAVEN EMERSON
Secretary: GEORGE M. MACKENZIE

Council:
GRAHAM LUSK
EUGENE F. DUBOIS
WALTER W. PALMER
H. D. SENIOR

1926–1927

President: WALTER W. PALMER   *Council:*
Vice-President: WM. H. PARK   GRAHAM LUSK
Treasurer: HAVEN EMERSON   HOMER F. SWIFT
Secretary: GEORGE M. MACKENZIE   A. R. DOCHEZ
                                                              ROBERT CHAMBERS

1927–1928

President: DONALD D. VAN SLYKE   *Council:*
Vice-President: JAMES W. JOBLING   GRAHAM LUSK
Treasurer: HAVEN EMERSON   RUSSELL L. CECIL
Secretary: CARL A. L. BINGER   WARD J. MACNEAL
                                                              DAVID MARINE

1928–1929

President: PEYTON ROUS   *Council:*
Vice-President: HORATIO B. WILLIAMS   GRAHAM LUSK
Treasurer: HAVEN EMERSON   ROBERT CHAMBERS
Secretary: PHILIP D. MCMASTER   ALFRED F. HESS
                                                              H. D. SENIOR

1929–1930

President: G. CANBY ROBINSON   *Council:*
Vice-President: ALFRED F. HESS   GRAHAM LUSK
Treasurer: HAVEN EMERSON   ALFRED E. COHN
Secretary: DAYTON J. EDWARDS   A. M. PAPPENHEIMER
                                                              H. D. SENIOR

1930–1931

President: ALFRED E. COHN   *Council:*
Vice-President: J. G. HOPKINS   GRAHAM LUSK
Treasurer: HAVEN EMERSON   O. T. AVERY
Secretary: DAYTON J. EDWARDS   A. M. PAPPENHEIMER
                                                              S. R. DETWILER

1931–1932

President: J. W. JOBLING
Vice-President: HOMER W. SMITH
Treasurer: HAVEN EMERSON
Secretary: DAYTON J. EDWARDS

Council:
GRAHAM LUSK
S. R. DETWILER
THOMAS M. RIVERS
RANDOLPH WEST

1932–1933

President: ALFRED F. HESS
Vice-President: HAVEN EMERSON
Treasurer: T. M. RIVERS
Secretary: EDGAR STILLMAN

Council:
GRAHAM LUSK
HANS T. CLARK
WALTER W. PALMER
HOMER W. SMITH

1933–1934

President: ALFRED F. HESS
Vice-President: ROBERT K. CANNAN
Treasurer: THOMAS M. RIVERS
Secretary: EDGAR STILLMAN

Council:
STANLEY R. BENEDICT
ROBERT F. LOEB
WADE H. BROWN

1934–1935

President: ROBERT K. CANNAN
Vice-President: EUGENE L. OPIE
Treasurer: THOMAS M. RIVERS
Secretary: RANDOLPH H. WEST

Council:
HERBERT S. GASSER
B. S. OPPENHEIMER
PHILIP E. SMITH

1935–1936

President: ROBERT K. CANNAN
Vice-President: EUGENE L. OPIE
Treasurer: THOMAS M. RIVERS
Secretary: RANDOLPH H. WEST

Council:
ROBERT F. LOEB
HOMER W. SMITH
DAVID MARINE

1936–1937

President: EUGENE L. OPIE
Vice-President: PHILIP E. SMITH
Treasurer: THOMAS M. RIVERS
Secretary: McKEEN CATTELL

Council:
GEORGE B. WALLACE
MARTIN H. DAWSON
JAMES B. MURPHY

1937–1938

President: EUGENE L. OPIE   *Council:*
Vice-President: PHILIP E. SMITH   GEORGE B. WALLACE
Treasurer: THOMAS M. RIVERS   MARTIN H. DAWSON
Secretary: MCKEEN CATTELL   HERBERT S. GASSER

1938–1939

President: PHILIP E. SMITH   *Council:*
Vice-President: HERBERT S. GASSER   HANS T. CLARKE
Treasurer: KENNETH GOODNER   JAMES D. HARDY
Secretary: MCKEEN CATTELL   WILLIAM S. TILLETT

1939–1940

President: PHILIP E. SMITH   *Council:*
Vice-President: HERBERT S. GASSER   HANS T. CLARKE
Treasurer: KENNETH GOODNER   N. CHANDLER FOOT
Secretary: THOMAS FRANCIS, JR.   WILLIAM S. TILLETT

1940–1941

President: HERBERT S. GASSER   *Council:*
Vice-President: HOMER W. SMITH   N. CHANDLER FOOT
Treasurer: KENNETH GOODNER   VINCENT DU VIGNEAUD
Secretary: THOMAS FRANCIS, JR.   MICHAEL HEIDELBERGER

1941–1942

President: HERBERT S. GASSER   *Council:*
Vice-President: HOMER W. SMITH   HARRY S. MUSTARD
Treasurer: KENNETH GOODNER   HAROLD G. WOLFF
Secretary: JOSEPH C. HINSEY   MICHAEL HEIDELBERGER

1942–1943

President: HANS T. CLARKE   *Council:*
Vice-President: THOMAS M. RIVERS   ROBERT LOEB
Treasurer: KENNETH GOODNER   HAROLD G. WOLFF
Secretary: JOSEPH C. HINSEY   WILLIAM C. VON GLAHN

1943–1944

President: HANS T. CLARKE   *Council:*
Vice-President: THOMAS M. RIVERS   ROBERT LOEB
Treasurer: COLIN M. MACLEOD   WILLIAM C. VON GLAHN
Secretary: JOSEPH C. HINSEY   WADE W. OLIVER

1944–1945

President: ROBERT CHAMBERS   *Council:*
Vice-President: VINCENT DU VIGNEAUD   WADE W. OLIVER
Treasurer: COLIN M. MACLEOD   MICHAEL HEIDELBERGER
Secretary: JOSEPH C. HINSEY   PHILIP D. MCMASTER

1945–1946

President: ROBERT CHAMBERS   *Council:*
Vice-President: VINCENT DU VIGNEAUD   PHILIP D. MCMASTER
Treasurer: COLIN M. MACLEOD   EARL T. ENGLE
Secretary: EDGAR G. MILLER, JR.   FRED W. STEWART

1946–1947

President: VINCENT DU VIGNEAUD   *Council:*
Vice-President: WADE W. OLIVER   EARL T. ENGLE
Treasurer: COLIN M. MACLEOD   HAROLD G. WOLFF
Secretary: EDGAR G. MILLER, JR.   L. EMMETT HOLT, JR.

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Dr. Samuel Graff

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Dr. William B. Langan
Dr. Alfred G. Langmann

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Dr. Henry D. Lauson
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Dr. Harry Le Veen
Dr. Louis Levin
Dr. Michael Levine
Dr. Philip Levine
Dr. Samuel Zachery Levine
Dr. Milton Levy
Dr. Robert L. Levy
Dr. N. D. C. Lewis
Dr. William H. Lewis, Jr.
Dr. Emanuel Libman
Dr. Charles C. Lieb
Dr. Seymour Lieberman
Dr. Geoffrey C. Linder
Dr. Fritz Lipmann
Dr. Arthur Livermore
Dr. David P. C. Lloyd
Dr. Robert F. Loeb
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Dr. Leo Loewe
Dr. Perrin H. Long
Dr. Warfield T. Longcope
Dr. L. G. Longsworth
Dr. Dorothy Loomis
Dr. R. Lorente de Nó
Dr. Mary Loveless
Dr. Alice Lowell
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CORRELATION OF NERVE ACTIVITY WITH POLARIZATION PHENOMENA[1]

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a. *Basic Facts and Terminology.*—Nerve physiology has been analyzed chiefly on the basis of (1) the results of measurements of differences of electric potential between points on the surface of the nerve and (2) the effects of electric currents applied to the nerve through electrodes on its surface. In all cases the interpretation of the experimental results requires the use of the theory of electrotonus.

The basic phenomenon of the theory is illustrated by diagram *I* of figure 1. An electric current supplied by a polarizing circuit is applied to the nerve through electrodes on its surface (p_1, p_2). Although the shape of the electrodes is rather unimportant, the situation is visualized best when one thinks of the electrodes as thin cylindrical bands surrounding the nerve. In the case of an homogeneous cylindrical conductor of the same diameter as the nerves ordinarily used in experimental work (about 1 mm.), the applied current would flow practically only in the segment of conductor extending between the polarizing electrodes; therefore, a recording instrument (*osc.*) connected in the manner indicated in figure 1, *I*, would not measure any potential difference during the flow of the applied current. In the case of nerve, however, even when the distance between electrodes p_1 and r_1 is made as great as 30–40 mm., the measuring instrument detects a potential difference which is usually called the *electrotonic potential*. On the other hand, if electrode r_2 is located at a great distance from p_1 and electrode r_1 is successively placed at a number of points of the p_1r_2 segment, it is found that the measured potential difference decreases in an exponential fashion when the p_1r_1 distance is increased.

These facts indicate that the nerve fibers are core conductors,

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i.e., cable-like structures (fig. 1, *II*), composed of an internal
longitudinal conductor or core, a membrane and an external
longitudinal conductor (interstitial tissue fluid). From these
three conductors the membrane has the lowest conductivity; it
plays the rôle of the insulating sheath of a cable and forces the
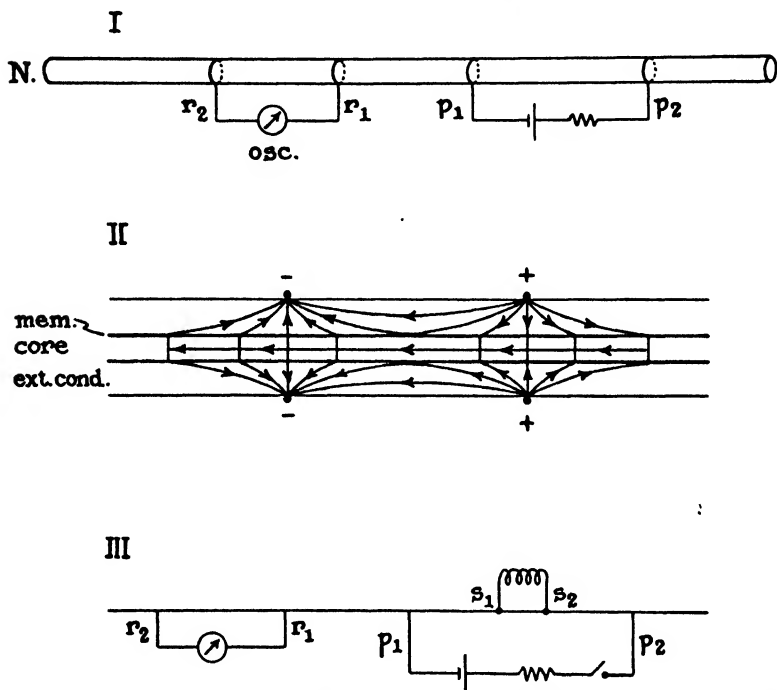

FIG. 1. Diagrams indicating the arrangement of electrodes and circuits
used in experiments on nerve.

applied current to spread along the nerve in the manner indicated
in figure 1, *II*.

Since the diameters of the nerve itself and of the individual
nerve fibers are small, the longitudinal conductors may be as-
sumed to be linear (linear in the sense of a geometrical line).
If it is further assumed that the external and the internal
longitudinal conductors have the properties of ohmic resistances

and that their resistances per unit length are r_e and r_i ohms respectively, an elementary analysis leads to the following important conclusion. Regardless of the nature of the membrane, the potential difference V_e measured on the surface of the nerve between electrodes r_1 and r_2 is related to the values V_1 and V_2 of the membrane potential at these two points by this simple formula:

$$V_e = \frac{r_e}{r_e + r_i} \; (V_2 - V_1)$$

Thus, if the membrane potential V_2 at r_2 is constant, the measured external electrotonic potential V_e is proportional to the change in the membrane potential V_1 at r_1.

In strict sense this simple interpretation of the external potential V_e is not correct since distributed electromotive forces appear in the core of the nerve fibers but it is adequate for the qualitative discussion to be made in this presentation. Indeed, within this narrow frame also the simplification is permissible to regard the nerve as containing only one nerve fiber (fig. 1, *II*).

In addition to the electrotonic potentials produced by applied currents, we will have to consider *demarcation potentials* arising from differences in the properties of adjacent segments of nerve and *action potentials* resulting from conduction of nerve impulses.

The simplest procedure to create a demarcation potential is to injure the nerve at one point, for example, by heating. In figure 2, *I*, the horizontal line *N* represents a nerve that had been injured at point *h* and the curve V_e, the demarcation potential measured at various distances from the injury. These differences, of course, were the result of the flow of demarcation current indicated in figure 2, *II*. Detailed analysis of the demarcation current and potential would necessitate consideration of an extensive experimental material. The main conclusion of the analysis, however, can be stated briefly. In resting uninjured nerve a difference of electrostatic potential exists between the internal longitudinal conductor and the interstitial tissue fluid; this potential difference is usually called the *membrane potential*. In the experiment illustrated by figure 2 a demarcation current flowed because the injury had destroyed the membrane and therefore the potential

difference between the core and the interstitial tissue fluid had been reduced to the value of a small junction potential. Demarcation currents also flow, of course, when the value of the membrane potential of a segment of nerve is altered by means of suitable chemical agents (figs. 5 to 7).
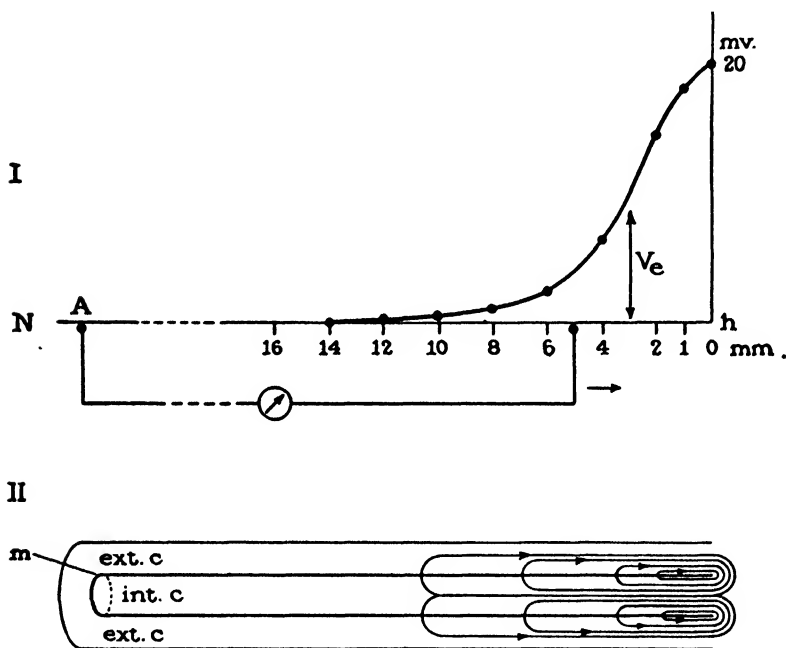

FIG. 2. *I*. Plot of the demarcation potential V_e against distance from the injured point *h*; *N*, nerve. *II*. Diagram indicating the distribution of demarcation current along the nerve; *m*, membrane; *ext. c.*, external longitudinal conductor, *int. c.*, internal longitudinal conductor (core).

The experimental arrangement ordinarily used to study action potentials is indicated in figure 1, *III*. A stimulating circuit (s_1, s_2) delivers to the nerve an electric shock of suitable strength which initiates a nerve impulse at the cathode (s_1). The impulse propagates itself along the nerve. It is clear that successive passage of the disturbance through points r_1 and r_2 must result in

the recording of a diphasic action potential (fig. 3, *2*) from which the potential changes at each electrode can be ascertained (fig. 3, *3*). If the distance between electrodes r_1 and r_2 is of the order of 2 or 3 mm., the diphasic record (fig. 3, *1*) can be regarded as the first derivative of the action potential; therefore, graphical integration can be used to determine the shape of the action potential. Ordinarily, however, the r_1r_2 distance is made relatively large and the nerve is injured at the level of electrode r_2 so that except for a relatively small artifact (fig. 20, *5, d.a.*) caused by the approach of the impulse to electrode r_2, the record gives directly the potential changes at electrode r_1. The diphasic artifact can be made negligible by treatment of the end segment of the nerve with KCl.

Analysis of the spike leads to the conclusion that during the conduction of the nerve impulse an alteration of the nerve fiber takes place which results in a sudden negative variation of the membrane potential. The recovery from the alteration and consequently the restoration of the membrane potential also takes place with great rapidity; however, each impulse leaves a small deficit of membrane potential which is restored at a relatively low rate. This residual depolarization is usually called the negative after-potential. The negative after-potential accumulates during conduction of trains of impulses (fig. 4). Under certain conditions the negative after-potential, after passing through a maximum, remains at a practically constant level even during 10 second tetani (fig. 4, *11, 12*); but under other conditions the negative after-potential continuously increases with increasing duration of the tetanus (fig. 4, *5, 6*). This second situation is theoretically very important since a progressive increase of the negative after-potential indicates that the nerve is passing into a state of depression or stated in a more familiar language, that the nerve is becoming severely fatigued (fig. 17).

The recovery from the residual depolarization ordinarily occurs through a temporary hyperpolarization of the membrane called the positive after-potential. Under certain conditions the positive after-potential includes a large R_3 deflection, under other
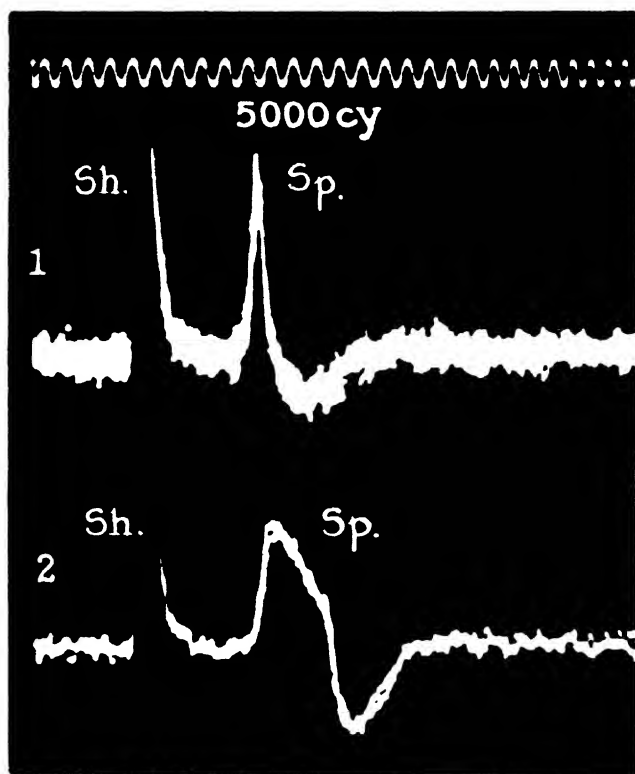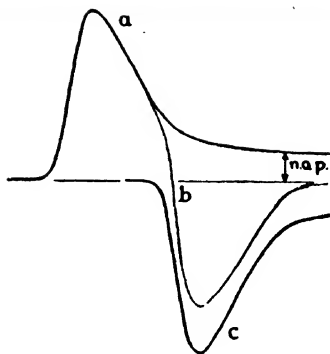
conditions, however, it may include only a brief R_2 deflection. In this presentation the term positive after-potential will be used only to denote the R_3 deflection.

b. *Membrane Potential.*—In the literature on nerve physiology it is customary to explain the resting membrane potential as a diffusion potential that results from the difference in the concentrations of potassium inside and outside the nerve fibers. This hypothesis was first enunciated by Bernstein (*cf.* Bernstein, '12) and although at present the hypothesis has several versions it is sufficient to discuss the original one which also is the most frequently used.

Bernstein's hypothesis is based on one fact and two assumptions. The fact is that the concentration of potassium inside the nerve fibers is much greater than the external concentration. According to modern estimates, the ratio of the two concentrations is 65:1 (*cf.* data by Fenn, Cobb, Hegnauer and Marsh in Gasser, '37). The first assumption is that the nerve membrane is semipermeable; it is permeable to potassium ions and impermeable to anions and to sodium ions. If the additional assumption is made that the membrane potential is maintained by outward diffusion of potassium ions, it is found that the value of the membrane potential would be proportional to the logarithm of the ratio of the internal and external concentrations of potassium, the factor of proportionality being 0.058 v. at 18° C.

Probably because the value calculated for the membrane potential is in agreement with experimental measurements, the diffusion hypothesis has received wide acceptance without having been submitted to more than perfunctory experimental tests. The hypothesis, however, cannot withstand a systematic analysis.

FIG. 3.   Spikes of single nerve fibers of the VIIth spinal nerve of a bullfrog recorded with electrodes 3 mm. apart (*1*) and 20 mm. apart (*2*). Both records are photographs of a number of successive oscillograph sweeps. Since the impulses were being propagated with constant speed, record *1* may be regarded as the first differential of the spike. *3*, reconstructions of the spikes at the first recording electrode (*a*) and at the second recording electrode (*c*) made on the basis of an enlarged tracing (*b*) of record *2*; *n.a.p.*, negative after-potential.   Nerve in 95% O_2 and 5% CO_2.

Figure 5 illustrates the effect of increasing the external concentration of K^+ ions upon the membrane potential of bullfrog sciatic nerve. The curves measure the demarcation potentials established between a segment of nerve in contact with Ringer's solution and another segment in contact with a mixture of Ringer's solution and an isotonic (0.11 M) solution of KCl. The detailed analysis of the demarcation potential curves is a difficult problem, but within the frame of this discussion it is sufficiently accurate to assume that the ordinates of the curves are propor-
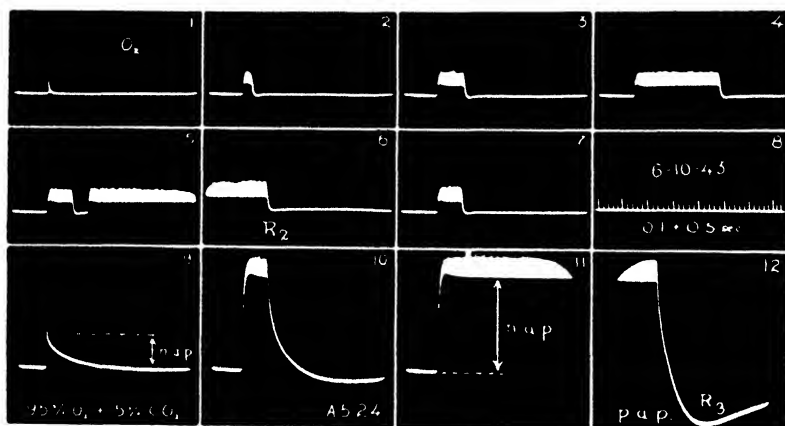

Fig. 4. After-potentials produced by single volleys of impulses (1, 9) and by rhythmic trains of impulses (2 to 7; 10 to 12); the trains used for records 5, 6, and 11, 12 were 10 seconds long. *n.a.p.*, negative after-potential; *p.a.p.*, positive after-potential. Records 1 to 7 were obtained with the nerve in oxygen and records 9 to 12 with the nerve in 95% O_2 and 5% CO_2.

tional to the decrease of the membrane potential of the segment of nerve in contact with the KCl solution.

The diffusion potential hypothesis predicts that if the external concentration of potassium is increased, the membrane potential of the nerve fibers will decrease. This prediction is in agreement with the experimental results presented in figure 5. In two essential points, however, the experimental results are in disagreement with the predictions of the hypothesis.
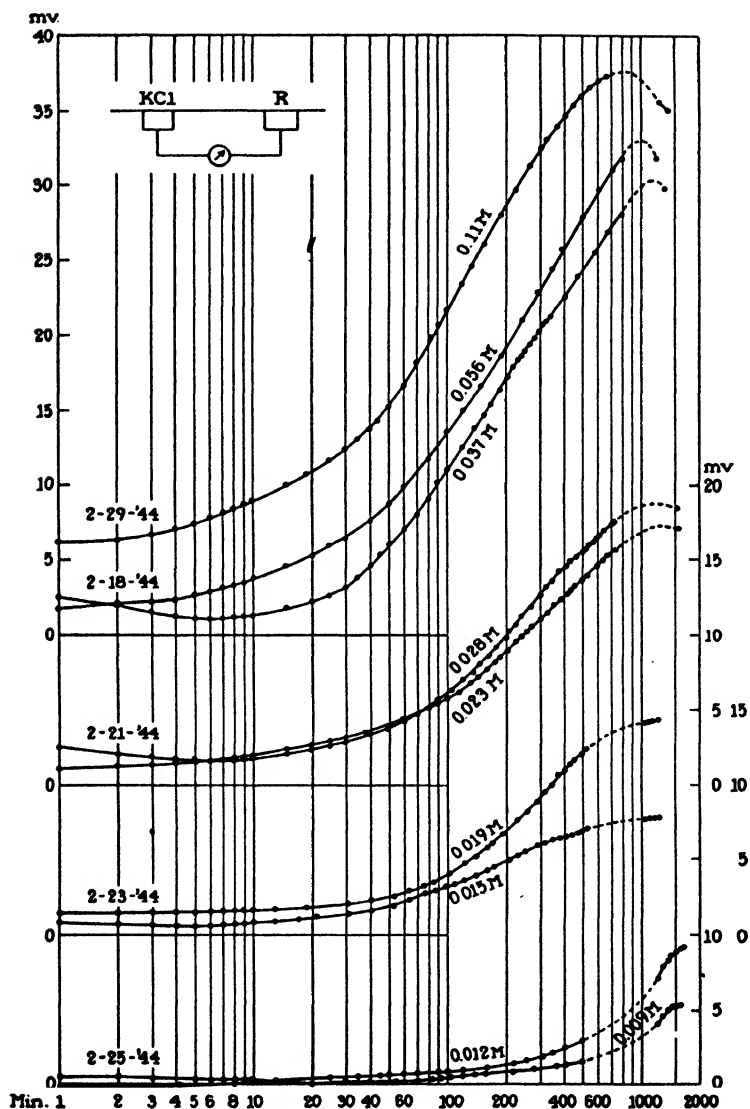
FIG. 5. Demarcation potentials resulting from the action of solutions of KCl upon a segment of the nerve. Ascent of the curves indicates depolarization of the treated segment.

According to the hypothesis, the decrease in the value of the membrane potential should take place immediately after the nerve is placed in contact with the KCl solution. In point of fact, however, the depolarizing action of KCl develops at an exceedingly low rate; even in the case of isotonic KCl (0.11 M) the depolarization does not approach its maximum until after the test solution has been allowed to act for several hours. It may be emphasized that according to the results of control experiments, the penetration of K^+ ions through the connective tissue sheath and the inter-fibrilar spaces up to the axis of the nerve is a rapid process; as a matter of fact, in relation to the slow depolarization of the nerve fibers, the penetration of K^+ ions into the nerve may be regarded as practically instantaneous.

The diffusion potential hypothesis also predicts that the decrease of the membrane potential should be proportional to the logarithm of the ratio of the new external concentration of K^+ ions to the normal concentration. In disagreement with this prediction the curves of figure 5 show that the depolarizations produced by the various test solutions were not on a logarithmic relationship to the external potassium concentrations at any arbitrarily selected time of action of the test solutions.

An assumption could be made in the attempt to conciliate the experimental observations presented in figure 5 with the predictions of the diffusion hypothesis. Nerves are known to gain potassium when the external potassium concentration is increased (Fenn, Cobb, Hegnauer and Marsh, '36); therefore it could be assumed that the membrane potential does not vary with the logarithm of the external potassium concentration because the internal concentration increases so that the ratio of the two concentrations undergoes only small changes. An argument like this, however, cannot be applied to the results of experiments of the type illustrated by figure 6.

A segment of nerve is placed in contact with 0.11 M KCl and after the depolarization has reached a significant value the KCl solution is replaced by a potassium-free solution. According to Bernstein's hypothesis, since the internal concentration of po-

tassium has been increased, the membrane potential should acquire a value in excess of the normal one immediately after the nerve has been placed in contact with the potassium-free solution; thereafter the membrane potential should decrease towards the normal value paralleling the outward diffusion of the excess of K^+ ions. These predictions are in sharp disagreement with the experimental results. The curves of figure 6 show that during the first
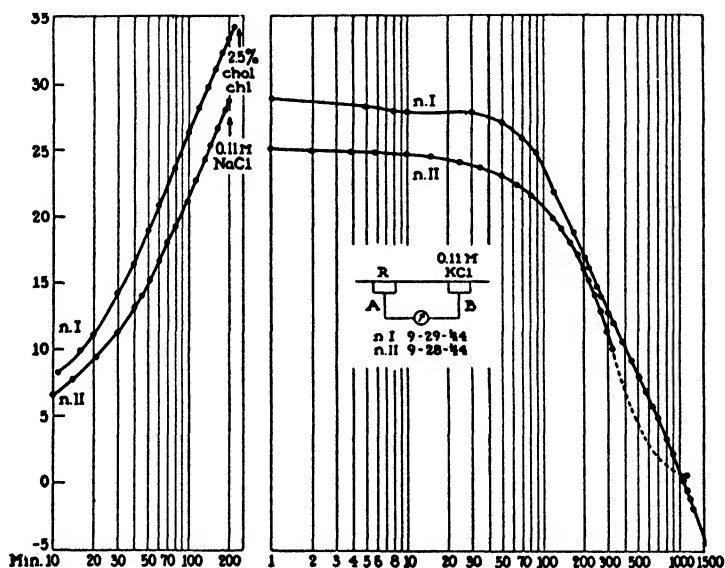
FIG. 6.   Depolarization of the nerve by isotonic KCl and repolarization in a potassium-free solution.

hour after the nerves had been placed in contact with the potassium-free solutions, the membrane potential remained practically constant at the low value that it had reached during the action of the excess of K^+ ions; thereafter a slow repolarization process began which required many hours for completion. The unavoidable conclusions to be drawn from the results of this experiment are: (1) that the value of the membrane potential is not directly dependent upon the ratio of the internal and external

concentrations of potassium and (2) that the membrane potential is not maintained by outward diffusion of potassium ions.

A number of years ago Koch ('27) and Gerard ('30) demonstrated that nerve deprived of oxygen undergoes a depolarization which is reversible; i.e., after oxygen is again made available to the nerve a repolarization takes place. In the past, this remarkable phenomenon has been placed in agreement with the diffusion potential hypothesis by assuming that the rôle of oxidative metabolism is only indirect. Metabolism would maintain the semipermeability of the membrane thereby creating those conditions under which outward diffusion of K^+ ions can establish the membrane potential. However, detailed study of the anoxic depolarization and the oxidative repolarization of nerve under a variety of experimental conditions leads to the conclusion that oxidative metabolism is the mechanism that directly creates the membrane potential. Complete presentation of the evidence cannot be made here, it will be convenient, however, to mention the results of one type of experiment that also yields a test of the diffusion potential hypothesis.

The three experiments illustrated by figure 7 were done with pairs of nerves. In all cases one half of the nerve was maintained in oxygen in contact with Ringer's solution while the other half of the nerve was placed in contact with a KCl solution, its atmosphere being oxygen for one nerve and nitrogen for the other nerve of the pair. The demarcation potential curves show that in all cases the combined effects of anoxia and excess of K^+ ions caused a greater depolarization than the excess of K^+ ions alone. However, after oxygen was admitted into the nerve chamber, the KCl-treated segment was able to perform an oxidative repolarization, its membrane potential rapidly becoming higher than that of the nerve kept in oxygen. Indeed, in two instances the oxidative repolarization temporarily brought the membrane potential above that of the segment of nerve in Ringer's solution. The detailed curves of the oxidative repolarization presented in the right half of figure 7 show the exceedingly high rate of the repolarization; in all cases the membrane potential reached its

maximum after 10–15 minutes of respiration. The brevity of this interval of time renders the assumption impossible that the repolarization was accompanied by significant changes in the
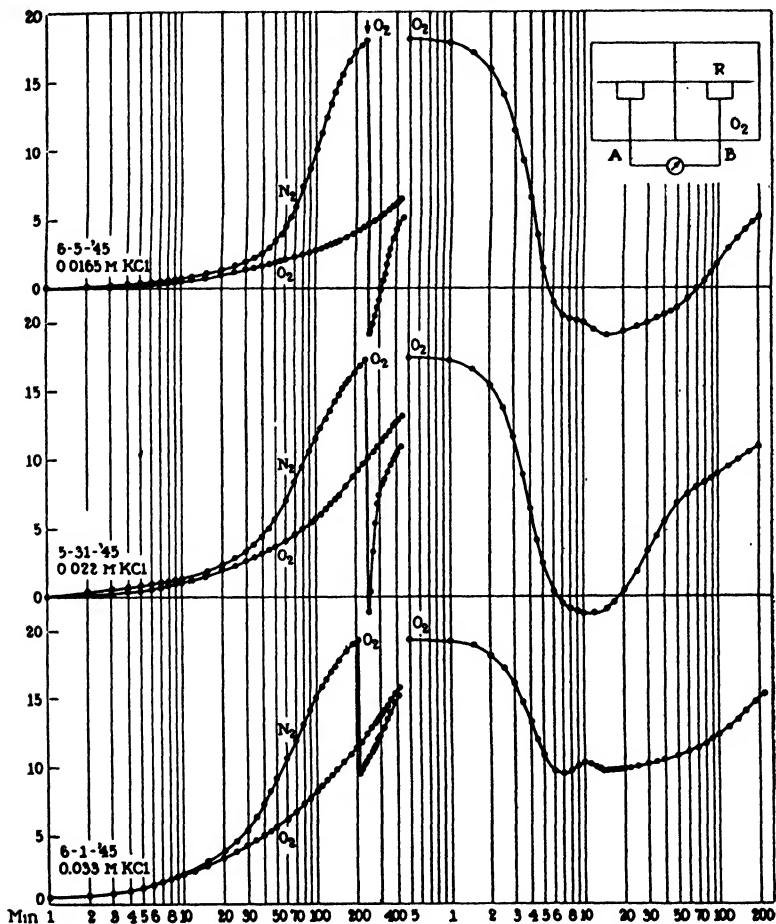

FIG. 7. Effect of an increased concentration of KCl upon the membrane potential of bullfrog sciatic nerve in the presence and in the absence of oxygen. The curves of the oxidative repolarization of the anoxic nerves have been reproduced in the right half of the figure on an extended time scale.

internal concentration of potassium; but regardless of any assumption that could be made in reference to the internal concentration of potassium there is no doubt that oxidative metabolism can counteract the effect of a large excess of K^+ ions in the external medium of the nerve fibers.

In view of these results and of others that cannot be mentioned in a brief report, there can be no doubt that the diffusion potential hypothesis must be discarded. The membrane potential is not referable to the existence of differences in the concentration of potassium inside and outside the nerve fibers; the membrane potential is directly established by oxidative metabolism.

The rôle played by potassium in nerve function is a matter for conjecture. The abundance of potassium inside the nerve fibers and the depolarizing action of an extracellular excess of K^+ ions decidedly suggest that potassium plays an important rôle; but however important this rôle may be, it is subordinate to that of oxidative metabolism.

Many assumptions could be made to account for the existence of a large amount of potassium inside the nerve fibers; among them there is one which is exceedingly interesting. In his Harvey Lecture Hastings ('40) described a drastic experiment to emphasize the importance of the intracellular ionic environment for the normal activity of intracellular enzymes. Making use or rather abuse of this concept, let us assume that potassium is present inside the nerve fibers at a high concentration simply because this concentration creates optimal conditions for the activity of the enzymatic systems of the nerve fibers. To be sure, an assumption like this is so exceedingly radical that it has little positive value. Nevertheless, the assumption is useful since consideration of it helps to discard from one's mind the diffusion potential hypothesis.

An often forgotten experimental fact can also be mentioned in order to emphasize that no greater importance for nerve function should be attached to potassium than to any other constituent of the nerve fiber. A frog nerve maintained in potassium-free Ringer's solution remains excitable for the same length of time

as a companion nerve maintained in Ringer's solution with the normal amount of potassium (usually 36 hours at 20° C.); a nerve, however, which is maintained in a sodium-free medium becomes inexcitable after 10–12 hours and regains its excitability when and only when Na^+ ions are made available to it (Overton, '02; Lorente de Nó, '44). If this experiment should be considered in isolation it would have to be concluded that for nerve function the most important ions are Na^+ ions.

When it comes to explain how oxidative metabolism establishes the membrane potential one finds that at the present state of knowledge, a detailed hypothesis cannot and should not be formulated. From the study of electrotonic potentials and action potentials, however, a general view is obtained that can serve (1) as a tool to integrate into a body of knowledge experimental observations of great diversity and (2) as a working hypothesis to predict the occurrence of phenomena which can be submitted to experimental analysis. Although the logical procedure would be to present first the evidence and then the working hypothesis, it will be simpler to describe first the hypothesis and then use it to discuss experimental results.

An important fact is that the membrane potential consists of several fractions; for the purpose of this report, however, it will be sufficient to consider only the two main fractions Q (quick) and L (labile). This division is justified by a number of experimental reasons among which the following is important. During the passage of the nerve impulse the membrane potential collapses. A part of the potential is restored during the descending limb of the spike while another smaller part is restored during the period of the negative after-potential. That part of the membrane potential which is restored during the descending limb of the spike is the Q fraction, and the other part which is restored during the negative after-potential is the L fraction. The division thus made is not entirely accurate since a small deficit of Q fraction remains after the end of the spike and a small part of L fraction is restored during the descending limb of the spike; fine details, however, need not be taken into account at this time.

Since the membrane potential is not a diffusion potential, it must be maintained at static double layers; a conclusion which is in full agreement with the results of polarization experiments
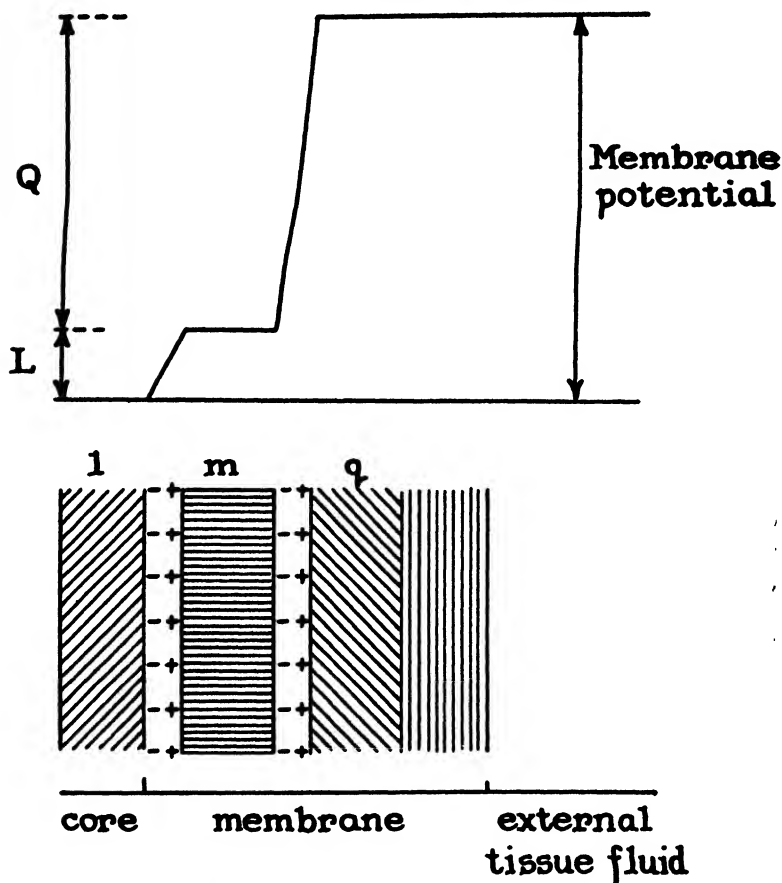

FIG. 8.   Diagrams illustrating the working hypothesis on the nature of the membrane potential.

(see later).   In view of the fact that the membrane potential consists of two fractions, it may be postulated that the membrane includes two boundaries (fig. 8).   The Q fraction is maintained

at a double layer at the boundary between the q and m phases and the L fraction at the boundary between the m and l phases. There are experimental facts to justify the assumption that the l phase is the internal one, it is perhaps the core itself.

In an important respect the differences of electrostatic potential Q and L which with resting nerve exist across the double layers represent equilibrium potentials. Since the charged particles of a double layer are under the influence of electrostatic (Coulomb) forces of attraction, the double layer could not exist unless non-Coulomb (chemical) forces should also exist at the boundary and should tend to separate charged particles of the opposite sign. Therefore, the value of the potential difference existing across a double layer is determined by the equilibrium of the Coulomb and non-Coulomb forces that act upon the electrically charged particles (*cf.* Helmholtz, 1847, 1853).

This situation can also be described in another manner. The transfer of a positively charged particle x and of a negatively charged particle y, for example, across the l–m boundary is a chemical reaction $lx, my = ly, mx$. Since the L double layer consists of x particles in the m phase and y particles in the l phase, it is clear that when this reaction proceeds in the direction $lx, my \rightarrow ly, mx$, the free energy of the system decreases. For this reason we may say that non-Coulomb or chemical forces act upon the particles x and y. On the other hand, the transfer of the charged particles across the boundary requires that a certain amount of work be done against the electric forces. If the decrease of free energy is greater than the electric work, the transfer of particles will take place in the indicated direction; if the decrease of free energy is smaller than the electric work, the transfer of particles will take place in the opposite direction; the equilibrium condition will be reached when the electric work and the decrease of free energy are equal.

It is thus clear that the resting or equilibrium value of the L fraction of the membrane potential is determined by the chemical reaction $lx, my \rightleftharpoons ly, mx$. On the other hand, a change in the value of the L fraction will drive the chemical reaction in one

direction or the other. In particular, if a short circuit is established between the external tissue fluid and the core so that a demarcation current can flow, the L potential will decrease since charged particles will be carried away from the double layer by the current; the double layer would rapidly disappear were it not that the decrease of the L potential allows the boundary reaction to proceed in the direction lx, $my \rightarrow ly$, mx so as to replace those charged particles which are carried away by the current. In view of this situation we may say that the non-Coulomb forces constitute the E.M.F. of the membrane. Similar considerations apply, of course, to the Q fraction of the membrane potential.

The rôle played by oxidative metabolism can now be easily described. The chain of reactions of the oxidative metabolism determines the chemical composition of the l, m and q phases and therefore creates the E.M.F. of the membrane. If the nerve is deprived of oxygen, the E.M.F. of the membrane decreases with the result that the double layers collapse. Postanoxic respiration reestablishes the E.M.F. of the membrane and therefore charged particles of the opposite sign are again separated into double layers. Since during the postanoxic repolarization the charged particles need be displaced only through distances of the order of magnitude of ionic radii, it is quite understandable that the repolarization can take place in a few minutes; it also is understandable that postanoxic repolarization can occur in the presence of a wide range of concentrations of potassium ions.

In the following discussion of electrotonic potentials the properties of the L fraction of the membrane potential will be analyzed first. Since the fluctuations of the value of the Q fraction take place at a much greater speed than the fluctuations of the L fraction, the fluctuations of the Q fraction cannot be photographed at the low sweep speed which is convenient for the study of the L fraction; they are included in the discontinuities of the records at the make and at the break of the applied current.

c. *Electrotonus and After-potentials.*—Ether anesthesia causes important changes in the electrotonus (Biedermann, '96, p. 694). In figure 9 the pairs of records *1* and *2* reproduce electrotonic

potentials in normal nerve, while the other pairs of records (*3* to *12*) reproduce the electrotonic potentials observed at the indicated intervals of time after the introduction of ether vapor into
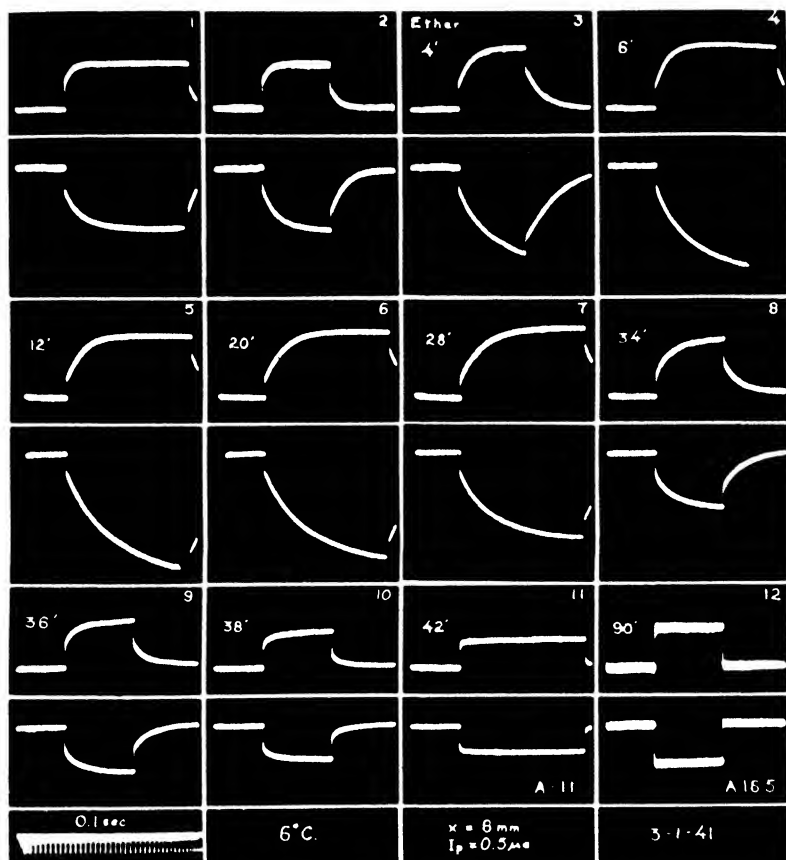

FIG. 9. Effect of ether anesthesia upon the electrotonic potential of bullfrog sciatic nerve. *A 11, A 16.5*, amplifications measured in millimeters of the original reproduction per millivolt input.

the nerve chamber. The partial pressure of ether was low during the first part of the experiment; it was increased in the interval between records *6* and *7*.

It will be noted in records *1* and *2* that the electrotonic potential displayed two components of widely different temporal course: (1) a fast component that appeared as discontinuities of the records at the make and at the break of the applied current and (2) a slow component that had a remarkably low rate of establishment and decay. The fast component of the electrotonus was modified relatively little by ether anesthesia; the slow component, however, underwent spectacular changes.

During the initial phase of the anesthesia (records *3* to *5*) the height of the slow component increased and the asymmetry of catelectrotonus and anelectrotonus became very pronounced (records *5*). During the second phase (records *6, 7*) the anelectrotonus decreased markedly while the catelectrotonus remained almost unchanged; consequently, the two potentials became symmetrical (records *7*). During the third phase (records *8* to *12*) the slow electrotonus decreased continuously; ultimately the electrotonic potentials displayed practically only fast components (records *11, 12,* note the higher amplification used for records *12*). At the slow sweep speed that was being used, the shape of records *12* was practically identical to the shape of records obtained by measuring the potential difference established by the applied current across an ohmic resistance which was included in the circuit in series with the nerve.

The effect of ether upon the electrotonic potentials is not specific; it is referable to the changes that ether causes in the value of the membrane potential. Initially ether increases the membrane potential, while later it causes a progressive depolarization. Any agent that increases the membrane potential causes an increase in the height of the electrotonic potential similar to that observed during the initial phase of ether anesthesia; likewise, any agent that causes depolarization of the membrane decreases the height of the slow electrotonus. In particular, it should be mentioned that electrotonic potentials of the type illustrated by records *11* and *12* of figure 9 also are observed with nerves that have undergone a far-reaching anoxic depolarization.

The difference in the behavior of the fast and the slow com-

ponents of the electrotonic potential clearly shows that the applied current establishes the two components by different mechanisms. Let it be assumed that the fast component which is recorded with depolarized nerve, measures the resistivity of the membrane.

The dependence of the slow electrotonus upon the value of the membrane potential suggests that the slow electrotonus is a polarization potential comparable to those which are measured by electrochemists at the electrodes of galvanic cells. Two main types of electrode polarization are considered by electrochemists, concentration polarization and chemical polarization or overpotential. The first mechanism can play only an insignificant rôle in the case of the slow component of the electrotonus since this component does not appear in nerves in the state of ether anesthesia nor in nerves that are conducting trains of impulses at frequencies above 40–50 per second (fig. 11, 6). Therefore the slow electrotonus, must belong to the group of overpotentials. As a matter of fact, there is evidence to show that the slow electrotonus consists in fluctuations of the L fraction of the membrane potential.

In the experiment illustrated by figure 10, a comparison was made at two different temperatures of (1) the electrotonic potentials produced by 30-second pulses of current, cathodal (electrode p_1, cathode) in the case of records 1, 2 and 9, 10 and anodal (electrode p_1, anode) in the case of records 3, 4 and 11, 12, and (2) the after-potentials produced by conduction of 30-second trains of impulses at the frequency of approximately 100 per second (records 6, 7, 14, 15).

If records 1, 2 are compared with records 6, 7 it will be found that the tracing of the catelectrotonus during the flow of the applied current and the negative after-potential during the tetanus, i.e., the height of the white band in records 6, 7, had the same temporal course; a lowering of the temperature to 27° C. modified both the catelectrotonus (records 9, 10) and the negative after-potential (records 14, 15); the change, however, was the same in the two instances.

After the end of the tetani the negative after-potential was replaced by the positive after-potential (records *7, 15*), i.e., by a hyperpolarization of the membrane; similarly, after the end of the applied currents the slow electrotonus reversed its sign (records *2, 10*). Thus, in the two instances the depolarization of
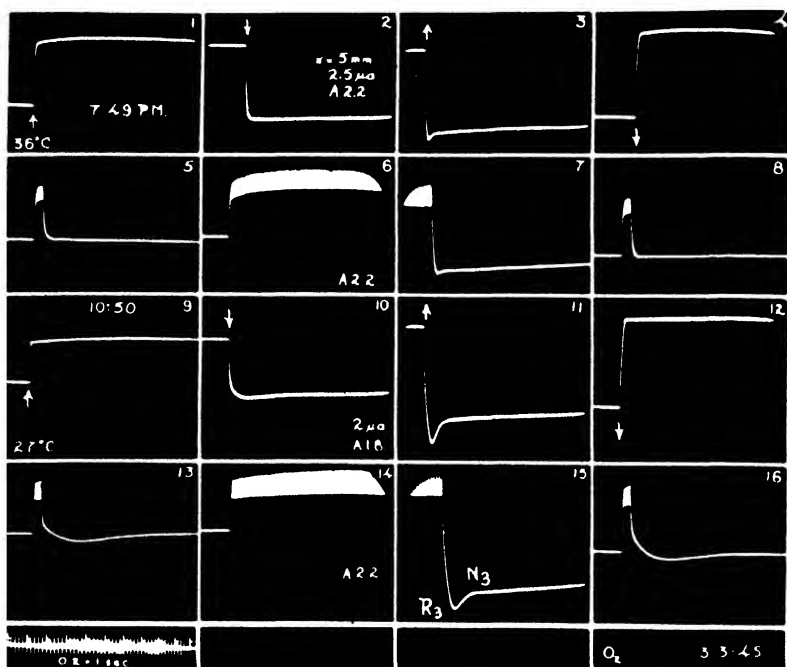
Fig. 10. Comparison of electrotonic potentials and after-potentials at 36° and at 27° C.

the membrane initiated a process that ultimately resulted in a hyperpolarization.

Although at the two temperatures the post-cathodal overshooting and the positive after-potential had similar temporal courses, the deflections in records *2, 7* and *10, 15* were not exactly identical. By properly choosing the magnitude and duration of the applied current and the duration of the tetanus it would have

been possible to produce identical post-cathodal overshootings and positive after-potentials.   In this experiment, however, the conditions were chosen so that the temporal course of the positive after-potential would be reproduced by the slow electrotonus created by the anodal current.   The experiment was successful, since the anelectrotonus (records *3, 11*) duplicated the positive
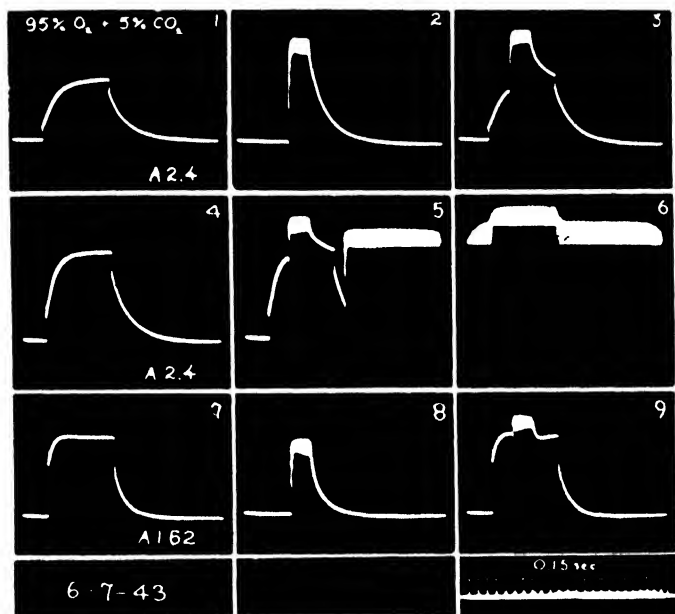
FIG. 11.   Effects of the superposition of pulses of cathodal current and of rhythmic trains of impulses.

after-potential (records *7, 15*) at the two temperatures.   Thus, we can reach an exceedingly important conclusion which also is in agreement with the results of a number of other experiments. The flow through the membrane of an applied anodal current produces changes in the membrane potential identical with those which are produced by the metabolic mechanisms of the nerve fibers during the recovery of the loss of membrane potential created by conduction of a train of impulses.

A convenient procedure to analyze the relationship of the slow catelectrotonus to the negative after-potential is to superpose the effects of pulses of applied current and rhythmic trains of impulses. In figure 11 record *1* presents the catelectrotonus produced by a rectangular pulse of current and record *2*, the negative after-potential produced by a brief tetanus. Record *3* presents the result of superposing the tetanus upon the applied current; as can readily be noted, the sum of slow catelectrotonus and negative after-potential in record *3* is equal to the negative after-potential in record *2*. Record *5* presents the result of superposing the tetanus of record *2* upon the catelectrotonus of record *4;* again the sum of slow catelectrotonus and negative after-potential in record *5* is equal to the negative after-potential in record *2*. The tetanus was recommenced during the sweep of record *5* and was continued through the sweep of record *6*. During the sweep of record *6* the rectangular pulse of current was superposed upon the tetanus; it produced only a rectangular deflection, the height of which was that of the fast component in record *4*. Thus, it can be concluded that the slow electrotonus and the negative after-potential represent the same change in the value of the membrane potential. The limiting value of both slow catelectrotonus and negative after-potential is that of the *L* fraction. This fact is shown again in records *7* to *9*. The current used to obtain record *9* was slightly greater than the rheobase of the nerve; since a current of this magnitude reduces the *L* fraction of the membrane potential to a very small value, the tetanus of record *8* produced only a very small negative after-potential when it was superposed upon the applied current (record *9*).

d. *The Nerve Reaction.*—In view of the fact that the slow electrotonus consists in changes in the value of the *L* fraction of the membrane potential, the mechanism of its production becomes readily understandable. It is clear that if the ions that carry the current were transported across the *L* double layer at the rate at which they reach the *l–m* boundary, the strength of the double layer and consequently the value of the *L* fraction would remain

constant during the flow of the applied current. Therefore, the slow electrotonus is a polarization potential of the same nature as the overpotential of electrodes (for modern theory of the overpotential *cf.* Gurney, '31, Butler, '40, Glasstone, Laidler, Eyring, '41). It is produced because the transport of ions across the *L* double layer is a relatively slow process and consequently the flow of the current is accompanied by changes in the strength of the double layer. An important consequence of this proposition is that the slow electrotonus is established without measurable changes in the concentration of ionized solutes; its establishment requires only a change in the number of charged particles in the two layers of the double layer and this change is far too small to be detected by the methods of analytical chemistry.

At the present state of knowledge no detailed assumption may be made regarding the process of transport of the current across the *L* double layer. Certain general features of the process, however, are understandable. With resting nerve the value of the *L* potential determines the equilibrium point of a chemical reaction at the *l–m* boundary insofar as the decrease of free energy that would result from the combination of charged particles of the *l* and *m* phases with constituents of the *m* and *l* phases respectively, is equal to the work that would be done in transporting those particles across the electrostatic potential difference existing at the boundary. When the slow electrotonus is established, i.e., when the value of the *L* fraction is changed, equilibrium ceases to exist at the *l–m* boundary so that the chemical reaction will progress in one or the other direction according to whether the slow electrotonus represents a decrease or an increase of the *L* potential. This situation is essentially identical to that which arises when the difference of potential between an electrode and a solution of its ions is changed. If the potential difference is changed in one direction the metallic cores go into solution as ions; if the electrode potential is changed in the opposite direction, the ions of the solution are deposited on the electrode.

Certain important details of the relationship of the electrotonus

to the magnitude of the applied current are illustrated by the series of records of figure 12. The magnitude of the current was varied from 0.03 μa (records *1* and *2*) to 3 μa (records *11* and
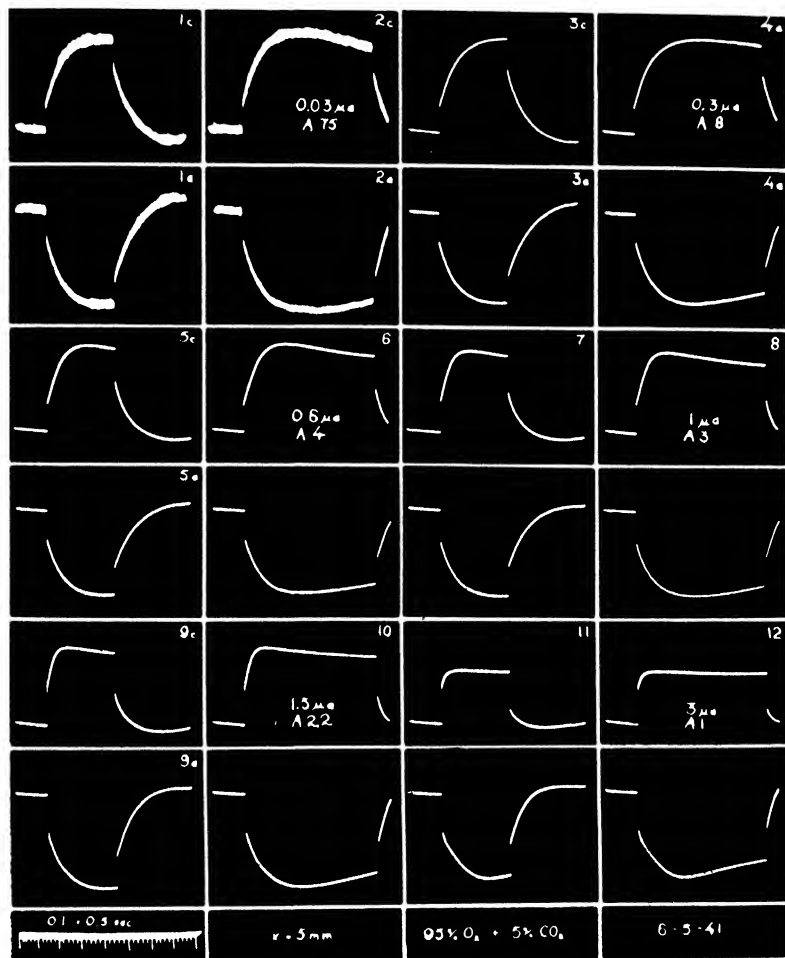

FIG. 12. Electrotonic potentials produced at 5 mm. from electrode p_1 by rectangular pulses of current of the indicated magnitude. Amplifications given in multiples of the amplification used for records 12.

12).   The latter current still was subliminal for a large majority of the fibers of the nerve; on the other hand, when the applied current was decreased to 0.01 μa and the amplification was increased, the electrotonic potentials still displayed essentially the temporal course that appears in records *1*.   The electrotonic potentials produced by smaller currents were of the order of magnitude of the tube noise of the amplifier; therefore, the series of records of figure 12 covers the whole practical range of subliminal currents.   Pulses of current of two different durations were used : short pulses in order to observe the overshooting after the end of the applied current (records of the first and third columns) and long pulses in order to observe the maximum of the potential during the flow of the applied current (records of the second and fourth columns).

If in examining figure 12 the amplification factors are taken into account it will be found that the fast component of the electrotonus was approximately proportional to the applied current while the relationship of the slow electrotonus to the applied current was markedly non-linear, with the noteworthy peculiarity that with small currents the catelectrotonus was higher than the anelectrotonus while with large currents the anelectrotonus was higher than the catelectrotonus.   There still are in figure 12 a number of details that deserve consideration; within the frame of this report, however, emphasis can be placed only upon these two, (1) in all cases the slow electrotonus passed through a maximum during the flow of the applied current and (2) in all cases the slow electrotonus reversed its sign after the end of the applied current.

Since the nerve fibers are core conductors, the theoretical analysis of the potential changes recorded at one point of the nerve offers considerable difficulties; the problem, however, becomes simple after the instantaneous distribution of the electrotonic potential along the nerve has been ascertained.   The technical procedure is not difficult, one determines experimentally the electrotonic potential at a number of points of the nerve and the experimental records are used to prepare families of curves such as those reproduced in figure 13.

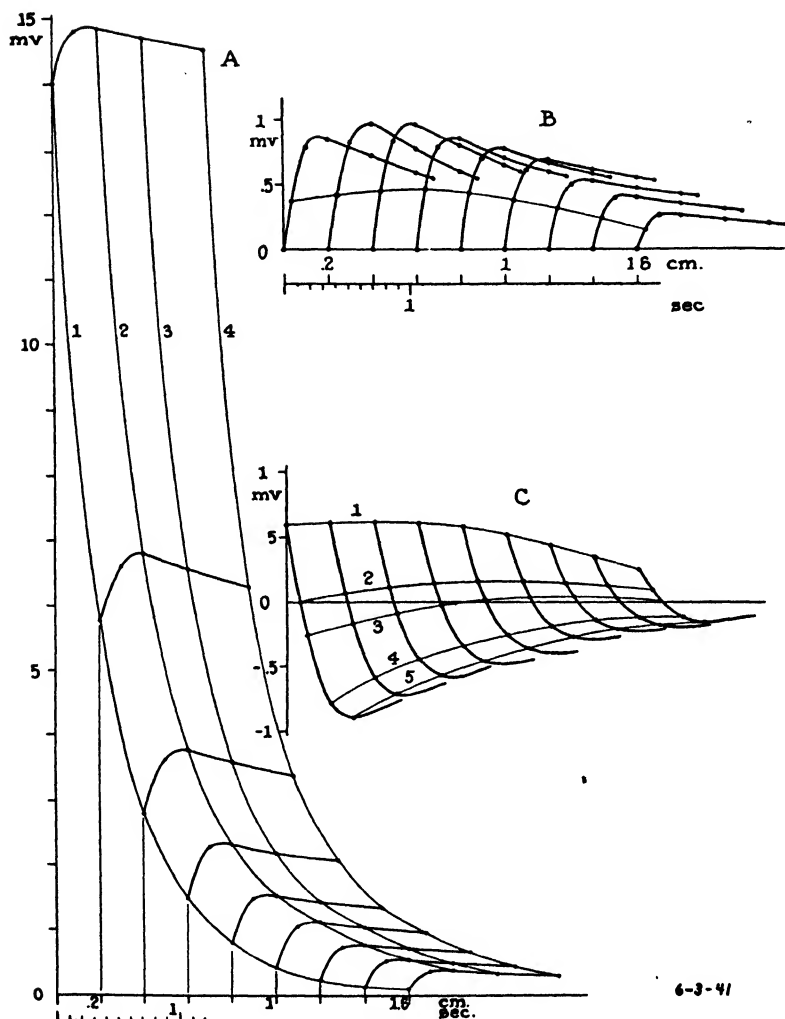FIG. 13. Plots of the temporal course and of the longitudinal distribution of the electrotonic potential in the extrapolar segment of the nerve. *A, B,* electrotonic potential during the flow of the applied current; *C*, electrotonic potential after the end of the polarization.   Curves *A* measure the total potential; curves *B* and *C* only the slow components.

Family of curves A figure 13 gives both the temporal course and the longitudinal distribution of the electrotonic potential during the flow of the current and family C the temporal course and longitudinal distribution of the slow electrotonus after the end of the applied current. Family A shows the remarkable fact that the electronic potential passed through its maximum almost at the same time at all points of a 16-millimeter segment of nerve. In addition, since the slope of the longitudinal distributions 1 to 4 is proportional to the current in the longitudinal conductors, it is clear that the decrease of the electrotonic potential below its maximum was accompanied only by negligible changes in the flow of longitudinal current.

Similar phenomena are illustrated by family of curves C. Since the slope of the longitudinal plots (1 to 5) is negligible, there can be no doubt that (1) the decrease of the slow catelectrotonus and the reversal of its sign took place almost simultaneously at all points of a long segment of the nerve and (2) the overshooting of the electrotonic potential occurred in the absence of a significant flow of longitudinal current. Under conditions such as these the only possible explanation of the overshooting is the following. At the end of the applied current the E.M.F. of the membrane had a greater value than in resting nerve, so that in the absence of the applied current the membrane potential increased beyond the value of the resting membrane potential. Later both the membrane E.M.F. and the membrane potential returned gradually to the normal value.

The fact that the E.M.F. of the membrane increased during the flow of the applied current also explains the production of the maximum of the electronic potential. On the one hand, the increase of the E.M.F. of the membrane resulted in the decrease of the electrotonic potential below its maximum and on the other, the increase of the E.M.F. of the membrane resulted in the establishment of a new condition at the polarizable boundary so that the electrotonic potential remained at a steady level during the further flow of the applied current. In the case of the anelectrotonus, the maximum and the overshooting of the electrotonic potential are referable to a decrease of the E.M.F. of the membrane.

Thus, analysis of the electrotonic potential reveals an exceedingly important fact. The nerve fiber has the ability of regulating the E.M.F. of its membrane in response to and to oppose impressed changes of its membrane potential. This process may be called the "nerve reaction." The action of a cathodal current is to decrease the membrane potential and the corresponding reaction of the nerve fiber consists in an increase of the E.M.F. of the membrane; similarly, the action of an anodal current is to increase the membrane potential and the corresponding reaction of the nerve fiber consists in a decrease of the E.M.F. of the membrane. In both cases the reaction tends to oppose the effect of the applied current so as to prevent a change in the value of the membrane potential. In view of the records presented in figure 12, it may be stated that the nerve reaction is a process that has no threshold of initiation; it is elicited by the flow of any current, however small.

Since the value of the electrotonic potential is determined by an action-reaction interplay, it should be expected that, at least under given conditions, the slow electrotonus would display oscillatory behavior. In point of fact, decremental oscillations of the slow electrotonus are frequently observed. A condition that favors their appearance is a subnormal value of the resting membrane potential.

Records *1* to *4*, *6* and *9* of figure 14 present examples of oscillatory behavior of the postcathodal overshooting. In addition, they illustrate the important fact that the duration of the applied current plays the rôle of an independent variable in the determination of the magnitude and temporal course of the overshooting. Records *11* and *12* present the behavior of the slow electrotonus during the flow of the anodal current. The similarity between records *6* and *11* is in agreement with the general principle according to which the processes established by the metabolic mechanisms in order to restore losses of membrane potential can be duplicated by applying to the nerve an anodal current. Finally, if record *7* is examined with some attention it will be found that during the flow of the cathodal current, the catelectro-

tonus passed through a series of oscillations of smaller magnitude but of the same frequency as those of the anelectrotonus (record 11).

As should be expected, when the postcathodal overshooting displays oscillatory behavior the positive after-potential also has an oscillatory course.
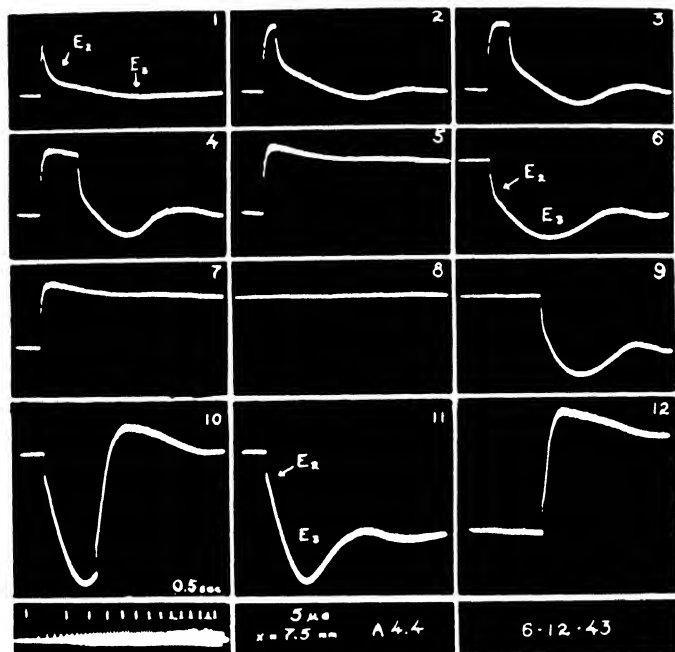
FIG. 14. Illustration of oscillatory behavior of the slow electrotonus.

e. *Slow Electrotonus and Excitability.*—An important feature of the slow electrotonus is that its fluctuations are accompanied by changes in the excitability of the nerve fibers. The relationship between the value of the L fraction of the membrane potential and the excitability of the nerve is so intimate that it can be defined in the following manner. Any change in the value of the L fraction causes a change in the excitability of the nerve

FIG. 15. Illustration of the relationship of the excitability of the nerve to the slow electrotonus.

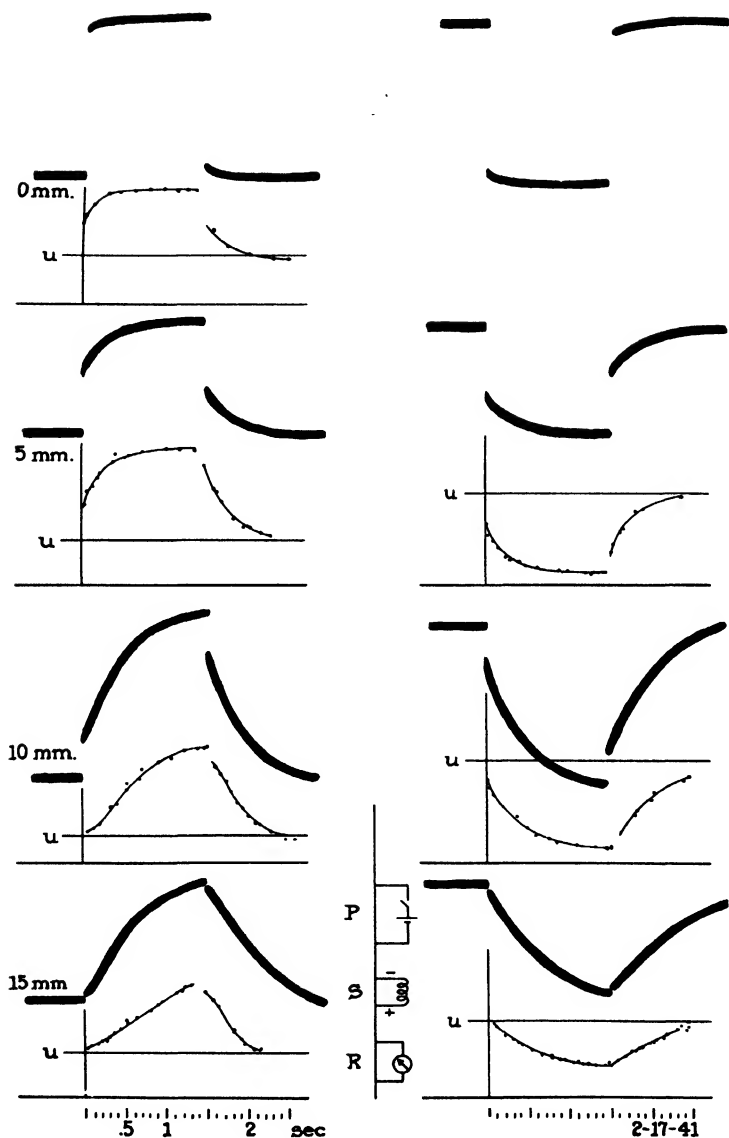fibers and conversely a change in the excitability of the nerve fibers, which takes place at the rate of the fluctuations of the slow electrotonus, may be taken as a sign that the value of the L fraction of the membrane potential is undergoing a change.

The curves and the tracings of records of electrotonic potentials reproduced in figure 15 illustrate the relationship between the excitability, i.e., the reciprocal of the stimulation threshold and the changes in the value of the L fraction of the membrane potential. The observations were made with the arrangement of electrodes indicated in the diagram, the distances between the polarizing electrode (p_1) and the cathode of the stimulating circuit (s_1) being those which are indicated on the left side of figure 15. In each case the electrotonic potentials were recorded at the stimulating cathode. As can readily be noted, the excitability curves faithfully paralleled the tracings of the slow electrotonus.

The relationship illustrated by figure 15 is generally true for nerves under ordinary experimental conditions, i.e., for nerves that have a membrane potential in the neighborhood of the normal value and are polarized at room temperature with applied currents of magnitude not exceeding more than a few times the rheobase of the nerve and of duration not exceeding a few seconds. Under these conditions a decrease in the value of the L fraction of the membrane potential causes a decrease in the threshold of stimulation regardless of whether the L fraction has been decreased by an applied cathodal current or by the operation of the E_3 reaction after the end of an applied anodal current (post-anodal overshooting). Conversely, an increase in the L fraction of the membrane potential causes an increase in the threshold of stimulation regardless of whether the L fraction has been increased by an applied anodal current or by the operation of the E_3 reaction after the end of an applied cathodal current (post-cathodal overshooting).

The relationship between the slow electrotonus and the excitability of the nerve fibers makes it possible to express Pflüger's well known rule in terms of directly measurable quantities.

Pflüger's rule is this, ''The establishment of the catelectrotonus and the disappearance of the anelectrotonus increase the excitability while the establishment of the anelectrotonus and the disappearance of the catelectrotonus decrease the excitability of the nerve fibers.'' In Pflüger's formulation the terms catelectrotonus and anelectrotonus denoted states of the nerve which heretofore could not be directly related to the value of the membrane potential. At present, insofar as the slow electrotonus is concerned, the situation can be accurately described in this manner: catelectrotonus is a state in which the L fraction of the membrane potential has a lower value than in resting nerve and anelectrotonus, a state in which the L fraction has a higher value than in resting nerve. In reference to the excitability of resting nerve, the excitability is increased in the catelectrotonic state and decreased in the anelectrotonic. This formulation would not have to be modified if catelectrotonus and anelectrotonus should be defined in reference to the resting blood-perfused nerve.

A rule has recently been given by Gasser ('37) to relate the excitability of the nerve fibers during the recovery after conduction of the impulses to the sign of the after-potential. ''The excitability of the nerve fibers is increased during the negative after-potential and decreased during the positive after-potential.'' An illustration of this relationship is presented in figure 16. Record *1* gives the height of the unconditioned testing spike. The nerve was submitted to continuous tetanic stimulation that was interrupted 3 seconds before the start of the sweep of record *16*. In addition, in order to test the excitability of the nerve fibers, the stimulation was interrupted shortly before or during each one of the sweeps of records *3* to *15*. Since all the records of figure 16 have the zero potential level in common, the differences between the heights of the base line of record *1* and of the base lines of the other records measure increments and decrements in the membrane potential. As can readily be noted, the excitability of the nerve was increased and consequently the testing spike was higher than in record *1* whenever the testing stimulus was applied during the negative after-potential, while the excitability was decreased during the positive after-potential.

A priori, one is inclined to think that Pflüger's and Gasser's rules apply to entirely different situations since Pflüger's rule describes changes in excitability produced by applied currents which may be subliminal while Gasser's rule describes changes in
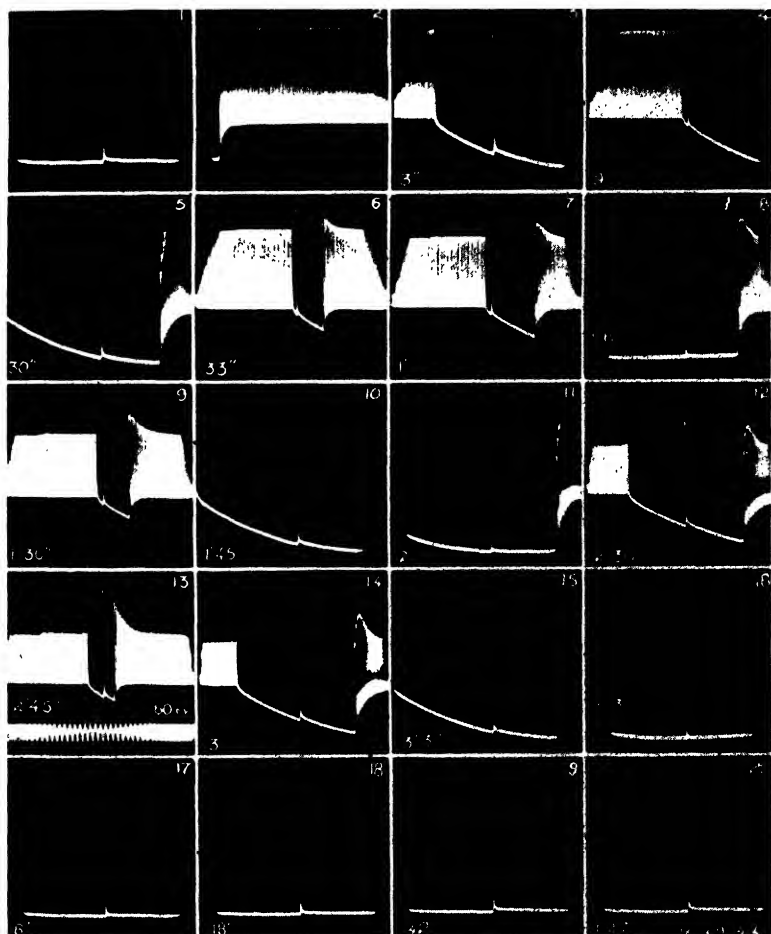

FIG. 16. Illustration of the relationship of the excitability of the nerve to the after-potentials in a nerve in which the *L* fraction of the membrane potential had been increased by the introduction of 5% CO_2 in the atmosphere.

excitability occurring during the recovery after conduction of impulses. However, if it is taken into account that under certain conditions the major part of the after-potentials consist of a change in the value of the L fraction of the membrane potential,

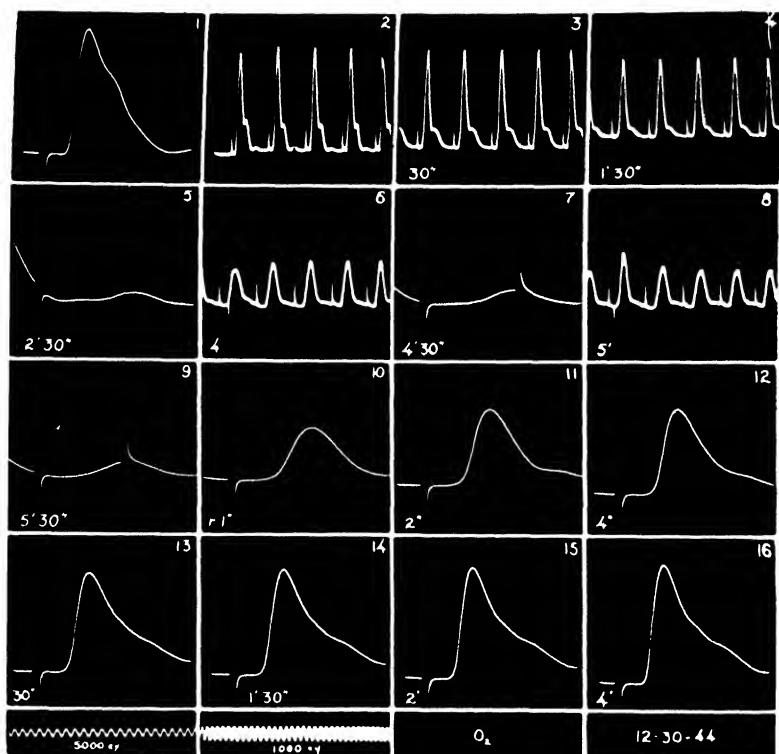
FIG. 17. Development of the state of cathodal depression by long lasting rhythmic conduction of impulses.

it becomes clear that under those conditions the excitability changes during the period of after-potentials must resemble the changes which accompany the slow electrotonus produced by subliminal currents. Therefore the contents of the rules of Pflüger and Gasser are essentially identical.

It should be emphasized that Pflüger's rule holds true when it is applied to changes in the membrane potential of nerves that are at or near to the normal state. There are, however, two important conditions under which the rule ceases to apply. If the nerve has been depolarized to such an extent that its excitability is depressed, the anelectrotonus will produce an increase of the excitability. Examples of this situation will be mentioned later. On the other hand, if with a nerve in the normal state, the applied cathodal current produces a catelectrotonus which includes a deficit of the Q fraction exceeding a certain limit, the catelectrotonus will cause a decrease of the excitability. This phenomenon was called in the classical literature "Werigo's cathodal depression."

The passage of an impulse leaves a deficit of Q fraction which is sufficient to produce cathodal depression or as it is usually called, relative refractoriness to stimulation. Under the experimental conditions that are used most frequently, the recovery of the deficit of Q fraction takes place quite rapidly so that the relatively refractory period is short but if the nerves are submitted to long-lasting tetanic stimulation, the cumulative loss of Q fraction may bring the nerve into a state of severe depression which may properly be called "fatigue." An example of this situation is presented in figure 17. Record 1 presents the spike elicited in the resting nerve by a supramaximal A shock and records 10 to 16, the spikes elicited by the same shock at the indicated intervals of time after the end of a rhythmic stimulation that lasted for 5.5 minutes. Records 2 to 4, 6 and 8 present the spikes of the conditioning tetanus which were initiated by shocks delivered through a second pair of stimulating electrodes. During the tetanus the fibers underwent a progressive loss of Q fraction, the extent of which is measured by the difference in the heights of the base lines of records 10 and 1; the slow rate of decay of the negative after-potential (residual depolarization) can be observed by noticing the downward displacement of the base line in records 10 to 16. Since in this case the negative after-potential consisted in a deficit of Q fraction, the excitability was

depressed as long as the negative after-potential was detectable. In addition, the spike height and the speed of conduction of impulses were subnormal.

The results of the experiment illustrated by figure 17 forcibly demonstrate that the interpretation of the excitability changes observed during the recovery after conduction of impulses must be made on the basis of this fact. The residual depolarization or negative after-potential left by the passage of nerve impulses includes two deficits, one of Q fraction and one of L fraction. A deficit of L fraction is always accompanied by a decrease of the excitation threshold while a deficit of Q fraction causes a decrease of the excitation threshold if it is small and an increase of threshold if it is large. The deficit of Q fraction left by the passage of an impulse always is sufficient to produce depression of excitability (relatively refractory period) but with nerves in which the L fraction is large, the effect of the deficit in L fraction may obscure the effect of the deficit in Q fraction. Indeed, the deficit in L fraction may be sufficient to lower the threshold below that of resting nerve.

Figure 18 illustrates the results of a reproducible experiment. All attempts to interpret these results met with failure until the work on the electrotonic potential had progressed so far that it became possible to postulate the existence of Q and L fractions in the membrane potential. Recovery curves I and II were obtained with the nerve in air immediately after excision, i.e., with the nerve in a state in which the L fraction of the membrane potential is very small and consequently the stimulation threshold is low. At this stage of the experiment the threshold of stimulation during the recovery was determined almost exclusively by the restoration of the deficit of Q fraction; therefore, no similarity existed between the recovery curve and the tracings of the after-potentials. After the nerve had been submitted to some experimentation the L fraction underwent a spontaneous increase so that when curves III and IV were obtained the effect of the deficit in the L fraction became demonstrable in the form of a shortening of the relatively refractory period. The atmosphere of the nerve was

then changed to 95% O_2 and 5% CO_2, a procedure that causes a large increment of the L fraction and therefore a large increase of the stimulation threshold. Under conditions such as these the threshold of stimulation during the recovery is determined mainly by the changes in the value of the L fraction, i.e., by the deficit
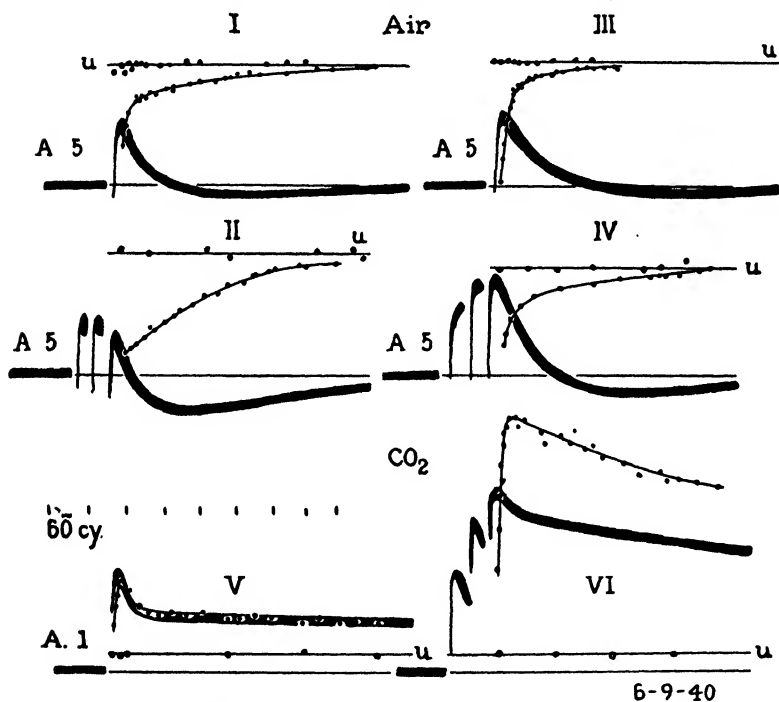
FIG. 18. Tracings of the after-potentials and curves of the recovery of excitability observed with the nerve in air (I to IV) and with the nerve in 95% O_2 and 5% CO_2 (V, VI).

of the L fraction during the negative after-potential and the excess of L fraction during the positive after-potential; consequently recovery curves V and VI paralleled the course of the negative after-potential. At the peak of curve VI the threshold of stimulation was approximately equal to the resting threshold at the time when curve I was obtained.

Between the extreme situations illustrated by curves *I, II* and *V, VI* of figure 18 there are a number of intermediate situations that can be created by judicious choice of the experimental conditions. A detailed discussion of the problem would overstep the frame of this report.

f. *Initiation of the Nerve Impulse.*—The problems that appear in the study of the initiation of the nerve impulse by applied currents are so many and of nature so varied that a complete analysis cannot be made within the frame of this report. The discussion will be limited to an analysis of the elementary process which initiates the nerve impulse. According to the available evidence this process is the same in all cases.

After a cathodal current has been applied to the nerve initiation of the nerve impulse occurs at the instant when the negative variation of the membrane potential reaches a certain value. However, the determining factor is not the total decrement of the membrane potential. In the first place the E_3 component of the catelectrotonus, i.e., the slow catelectrotonus, by itself never initiates an impulse. Indeed, even the total removal of the L fraction by itself does not result in the initiation of impulses. On the other hand, the applied cathodal current usually initiates impulses before it has been able to remove more than a small part of the L fraction and, under given conditions, the impulse may be initiated in the presence of an L fraction increased to the extent that the total value of the membrane potential is greater than in resting nerve. The initiation of the nerve impulse is referable to a component of the catelectrotonus which has a more rapid temporal course than the E_3 component; it will be called the E_1 component.

Figure 19 presents at two different sweep speeds the electrotonic potentials produced by rectangular pulses of cathodal current (cathode, electrode p_1) of progressively increasing duration. The pulse used to obtain records *13* and *14* was 2 seconds long. In all those records of figure 19 which were obtained with pulses of sufficient duration the electrotonic potential displayed a maximum during the flow of the applied current and an overshooting after

the end of the current. The maximum and the overshooting of record *6* of figure 19 are quite similar to the maximum and the overshooting in record *5c* of figure 12; in view of the fact, however, that the time scales used in the two instances were nearly in the ratio 500:1, it is obvious that the fluctuations of the
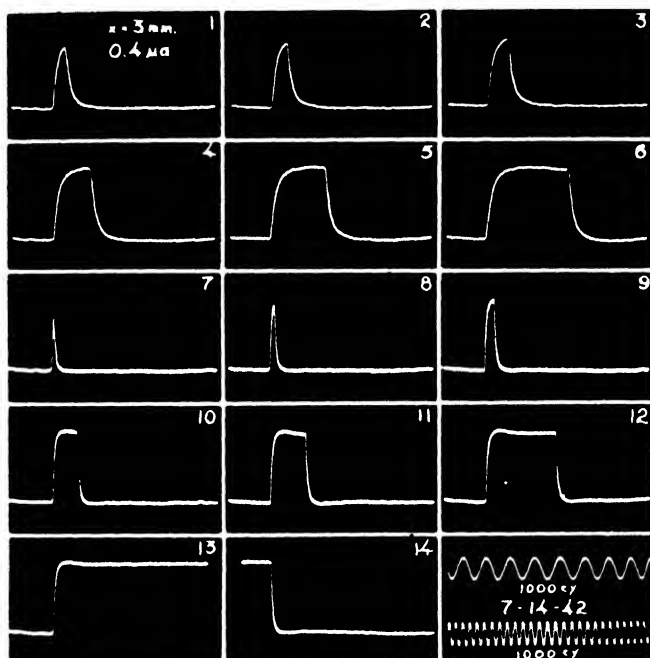
FIG. 19. Catelectrotonic potentials produced at 3 mm. from electrode p_1 by a subliminal current.

electrotonic potential must have been caused by different processes. The fluctuations that appear in the records of figure 19 were included in the discontinuities of the records of figure 12, i.e., in the "fast electrotonus."

In view of the existence of maxima and overshootings, it is clear that the fast electrotonus cannot be referable solely to the flow of current through layers of the membrane which have a low

conductivity. Since anesthesia, anoxia, lack of Na^+ ions, and a number of chemical agents cause the disappearance of the fluctuations of the fast electrotonus, it is reasonable to conclude that the fast electrotonus includes an F component that, loosely speaking, may be said to measure the resistivity of the membrane and an E_1 component which is a polarization potential comparable to the E_3 component or slow electrotonus. Unfortunately, no experimental procedure has been found that could lead to a satisfactory estimate of the relative heights of the F and E_1 components of the fast electrotonus. The evidence, however, leaves no doubt that the value of the E_1 component also is the result of an action-reaction interplay.

The applied current produces a polarization potential which is a change in the value of the Q fraction of the resting membrane potential. This change initiates a response, the E_1 reaction which results in a change of the E.M.F. that maintains the Q potential at the q–m double layer. The change in the E.M.F. is such as to oppose the change in the Q potential, that is to say, when an applied cathodal current decreases the Q potential, the E_1 reaction increases the E.M.F. of the membrane, while, when an applied anodal current increases the Q potential, the E_1 reaction decreases the E.M.F. of the membrane. In either case the nerve reaction removes part of the polarization potential created by the current and changes the conditions at the q–m boundary so that further flow of the current does not increase the polarization potential. In other words, the E_1 reaction is a process by means of which a dynamic equilibrium is established between the applied current and the nerve fiber. When the equilibrium is broken because the applied current is interrupted, the changed value of the E.M.F. of the membrane results in an overshooting of the electrotonic potential.

The significance of the E_1 reaction becomes apparent when it is observed that passage of nerve impulses initiates the same fluctuations of the Q fraction of the membrane potential as the flow of an applied current, therefore, the E_1 reaction which causes the overshooting after the end of a cathodal pulse is precisely

that process by means of which the activity of the metabolic mechanisms tends to restore the loss of Q potential left by the passage of the nerve impulse.[2] On the other hand, it can be shown that the flow of an applied current duplicates the effect of the activity of the metabolic mechanisms.

Figure 20 presents records of the spike of an alpha volley of impulses at three different sweep speeds and at two amplifications. Since the second recording electrode (r_2) was on a point of the nerve that had only been injured by sharp crushing, the record was the result of the superposition of the spike at electrode r_1
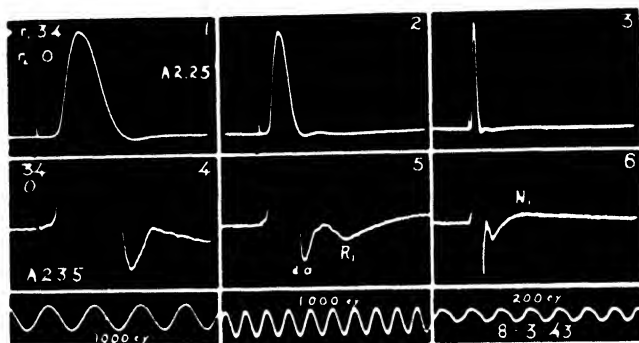

FIG. 20. Illustration of the diphasic artifact, $d.a.$ and the R_1 deflection of the spike.

(point 34) and a small change of potential at electrode r_2 (point 0). This change produced that positive phase of the spike which is usually called the diphasic artifact (fig. 20, 5, $d.a.$). The artifact, however, was followed by another positive deflection,

[2] The fact that the fluctuations in the value of the Q fraction take place with great rapidity seems to be in contradiction to the fact that a deficit of Q fraction may persist for a considerable period of time during which the nerve is in a state of depression (fig. 17). The explanation of the discrepancy is that the operation of the E_1 reaction is sufficient only to restore small losses of Q fraction, while the restoration of greater losses involves as a preliminary step the creation of an increment of the L fraction, a part of this increment being converted at a relatively slow rate into Q fraction. Presentation of the evidence that is available to support this conclusion cannot be made within the frame of a brief report.

R_1, that denoted a temporary increase of the Q fraction at point
34.   Ordinarily the R_1 deflection is submerged in the diphasic
artifact.   In this experiment the artifact and the R_1 deflection
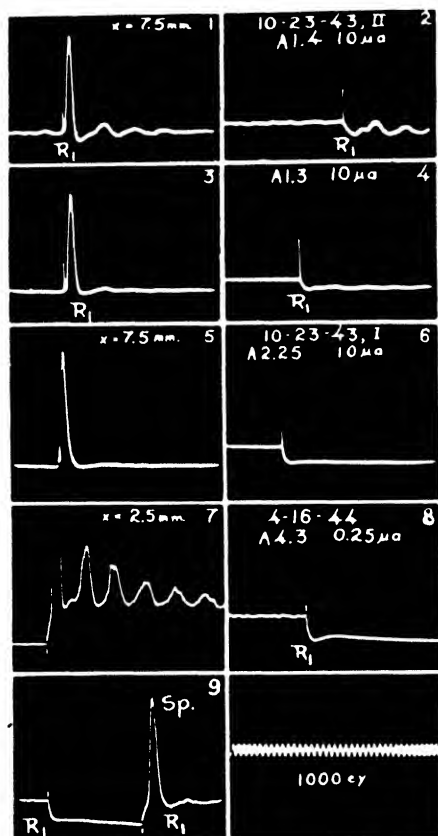

FIG. 21.   Illustration of the similarity of the fluctuations of the membrane
potential produced by the passage of a volley of impulses and by applied
currents.

were separated by choosing the r_1r_2 distance so that the R_1 de-
flection would follow after the artifact.   Another procedure that
can be used to demonstrate the existence of the R_1 deflection is to

treat the end segment of the nerve with KCl so as to prevent changes in the potential of electrode r_2. Since thereby the appearance of the diphasic artifact is prevented, the R_1 deflection is recorded without distortion. This procedure was used to obtain the records of figure 21.

In the experiments illustrated by figure 21, *1 to 6,* a comparison was made of the R_1 deflection produced by the passage of the nerve impulse and the overshooting of the electrotonic potential after the end of applied cathodal currents. The state of the oxalate-treated nerves was changed by removing the oxygen from the atmosphere. The similarity between the behavior of the membrane potential after the spikes and after the end of the cathodal currents was striking. In the case of records *1* and *2* the R_1 deflection was followed by rhythmic fluctuations of the membrane potential accompanied by firing of impulses; lack of oxygen modified the R_1 deflection and the following fluctuation of potential in the same manner (records *3, 4*); finally, in a more advanced stage of anoxia, both the spike and the cathodal pulse of current were followed only by faint R_1 deflections (records *5, 6*).

Records *7* and *8* were obtained also with an oxalate-treated nerve with the use of a 2-second pulse of current. Record *7* illustrates an important phenomenon. The flow of the applied current initiated a rhythmic discharge of impulses, each spike starting immediately after the R_1 deflection of the preceding spike. The explanation of this phenomenon is the following. The R_1 deflection actually is the first half-wave of a decremental oscillation; often it is followed by a sharp negative crest, N_1 (fig. 20, *6*), and not rarely the R_1–N_1 sequence is followed by decremental oscillations, the intervals between successive negative crests being about 5 to 8 msec. at 20° C. If the membrane potential is low, impulses are initiated at or shortly before the N_1 crest, the stimulus for the initiation of the impulse being the variation of the Q fraction that occurs during the transition from the R_1 deflection to the N_1 crest. The rhythmic discharge is therefore self-maintained in so far as each impulse supplies

the stimulus for the initiation of the following one. The fundamental frequency of the discharge is that of the R_1-N_1 oscillation; slower rhythms, however, can also appear since modulation of the R_1-N_1 oscillations by slower potential fluctuations may result in the initiation of impulses at the second or third N_1 crest instead of at the first.

The rhythmic discharge ceased during the interval between records 7 and 8 so that record 8 presents only the R_1-N_1 sequence after the end of the cathodal pulse. R_1 deflections also are present in record 9 which was obtained by the use of a short pulse of anodal current. There is in record 9 an R_1 deflection immediately after the make of the anodal current and another R_1 deflection following after the spike that was initiated by the break of the current. Thus, record 9 illustrates two important facts: (1) the flow of an anodal current produces an E_1 fluctuation the sign of which is opposite to that of the E_1 fluctuation at the make of the cathodal current and equal to that of the E_1 fluctuation after the break of the cathodal current, and (2) the flow of an applied anodal current initiates that sequence of potential changes which occurs during the recovery of the deficit of Q potential left by the passage of the nerve impulse; i.e., the flow of the anodal current duplicates the effect of the activity of the metabolic mechanisms also in the case of the E_1 component of the electrotonus.

The E_1 fluctuations of the electrotonic potential are accompanied by large changes in the excitability of the nerve fibers. In the experiment illustrated by figure 22 a comparison was made of the electrotonic potentials and the excitability of the nerve fibers. Curves Ic and IIc present the excitability changes at the make (Ic) and after the break (IIc) of the cathodal current and curves Ia and IIa, the excitability changes at the make (Ia) and after the break (IIa) of the anodal current. Curves $IIIc$ and $IIIa$ present with a slower time line the excitability changes during the flow of the cathodal ($IIIc$) and of the anodal ($IIIa$) currents. The tracings labelled P present the fluctuations of the electrotonic potential. As can readily be noted, the

excitability curves faithfully paralleled the E_1 fluctuations of the electrotonic potential.

The excitability changes illustrated in figure 22 also are in agreement with Pflüger's rule. If in reference to the E_1 com-
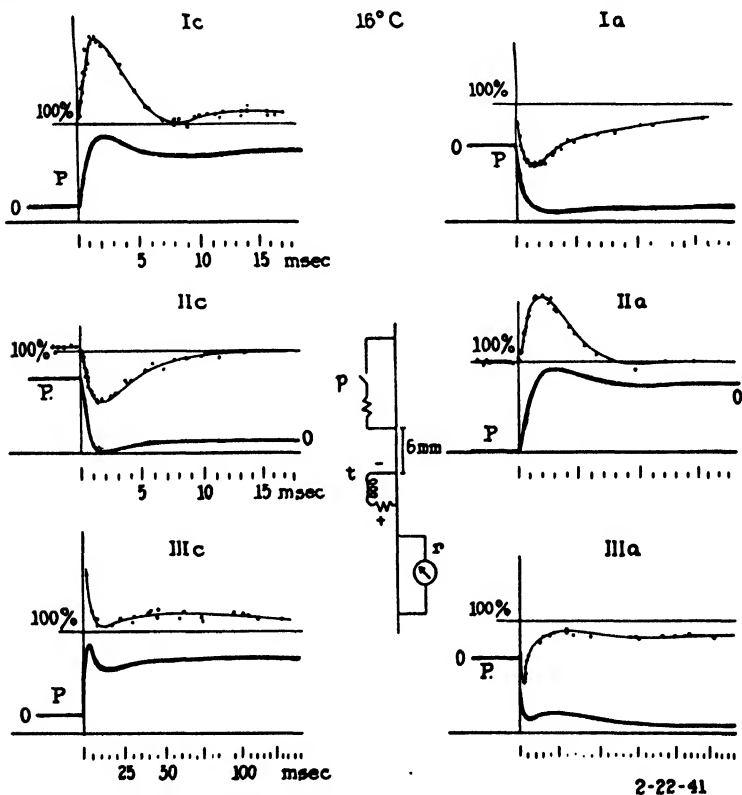
FIG. 22. Relationship of the excitability of the nerve to the E_1 fluctuations of the electrotonic potential.

ponent of the electrotonus, catelectrotonus and anelectrotonus are defined as states in which the Q fraction of the membrane potential is decreased and increased, respectively, Pflüger's rule can be stated in this manner. The excitability is increased in the catelectrotonic and decreased in the anelectrotonic state regard-

less of whether the change in the Q fraction has been produced directly by the applied current or by the operation of the E_1 reaction after the end of the applied current. It should never be forgotten, however, that if the decrease of the Q fraction exceeds a certain limit, the excitability becomes depressed.

There is a considerable body of experimental evidence leading to the conclusion that the creation of negative E_1 potential is the mechanism by which applied currents initiate the nerve impulse. Particularly significant are the following facts. If the magnitude of a cathodal current is barely sufficient to reach the threshold of the nerve fibers, the impulses are initiated at the crest of the E_1 fluctuation, never later; similarly, when the impulses are initiated by the break of the cathodal current, they arise at the N_1 crest which follows after the R_1 deflection. Impulses can also be initiated by the make of the anodal current; the necessary condition being that the nerve be so near to the rhythmic state that the E_1 potential displays an oscillatory course. Under these conditions the make of an anodal current of small magnitude initiates oscillations of the E_1 potential, impulses arising at the negative crests of the oscillations (fig. 23, *2* to *12*). If the magnitude of the applied current is increased, the value of the membrane potential is rapidly raised to a level at which E_1 oscillations cannot take place, whereby the initiation of impulses is prevented (fig. 23, *13* to *15*). It is therefore clear that in the case of small anodal currents the impulses are not initiated directly by the current, they are initiated by the operation of the E_1 reaction which creates a negative variation of the Q fraction.

The nature of the accommodation of the nerve to the cathodal current is now readily understandable. Accommodation is the result of the operation of the E_1 reaction. The applied current creates the E_1 potential while the E_1 reaction first opposes the growth of the E_1 potential and then removes a significant part of the E_1 potential that has already been created. If, at the time when it passes through its maximum, the E_1 potential does not reach the threshold of the nerve fiber, the impulse will not
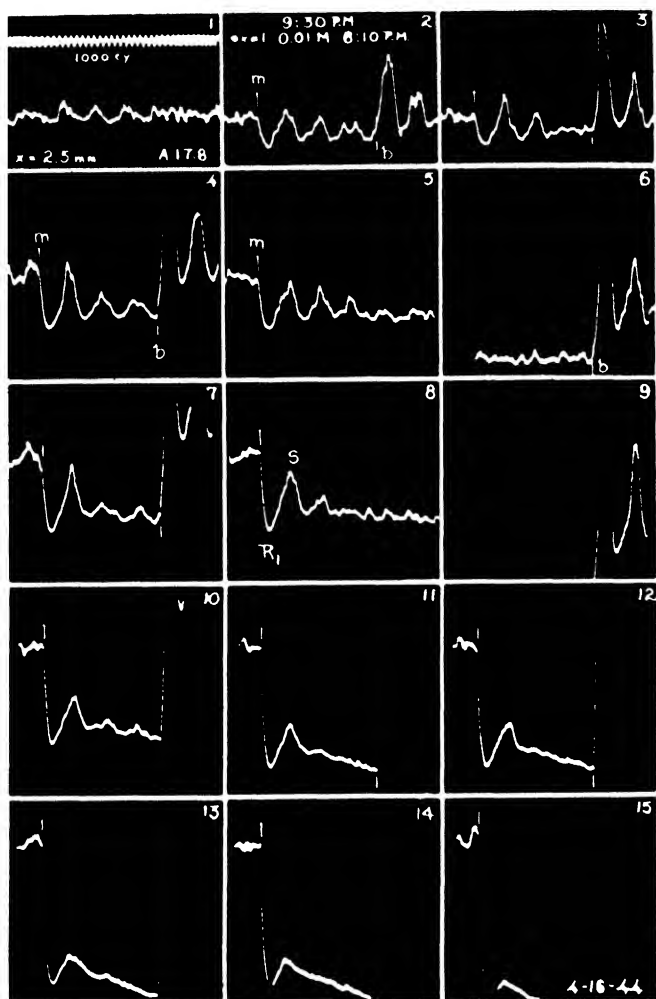
Fig. 23. Effect of anodal currents of progressively increasing magnitude upon oxalate-treated nerve shortly after the onset of spontaneous rhythmic activity. *1*, the base line in the absence of stimulation. The make and the break of the current are indicated by short vertical lines labelled *m* and *b* in several records. The *R₁* deflection has been labelled in record *8; s* in this record denotes the spikes initiated by the operation of the nerve reaction.

be initiated since the E_1 reaction changes the conditions at the q–m boundary in such a manner that further flow of the current will not produce an increase of the E_1 potential. Thus, according to this view, accommodation is the result of an active process, the E_1 reaction, which removes an important fraction of the stimulating agent, the E_1 potential, created by the current.

It may be of interest to mention that the hypothesis of the "local cathodal response" (Katz, '37), recently put forward to explain the initiation of the nerve impulse, is untenable. The hypothesis states that the nerve fiber acts as a passive structure for anodal currents of any magnitude and for cathodal currents of magnitude below 50% of the rheobase, while cathodal currents of greater magnitude initiate an active "local response" consisting of a depolarization which adds itself to the depolarization produced by the current. All parts of this hypothesis are erroneous. (1) The nerve fibers never act as passive structures; they produce a response to any current, cathodal or anodal, however small, and (2) the response that they produce is precisely the opposite of that which is assumed in the hypothesis of the "local cathodal response." The response of the nerve fibers, the nerve reaction, opposes the effect of the applied current. If the nerves of invertebrates were comparable at all to vertebrate (frog, mammalians) nerves, Hodgkin's ('38) hypothesis of the "local cathodal response" also would have to be discarded.

Let this point be made perfectly clear. Cathodal currents produce two essentially different responses in vertebrate nerve. The response to subliminal currents is the nerve reaction that opposes the effect of the applied current. If the magnitude of the current is increased so that the nerve reaction becomes insufficient to oppose its effect, a nerve impulse is initiated. Thus, there is a sharp discontinuity between the effects of subliminal and of liminal cathodal currents. Even more, there is a sharp discontinuity in the response of the nerve fiber to a liminal current; before the impulse is initiated, the nerve fiber opposes the depolarizing action of the current while, when the nerve impulse arises, the nerve fiber undergoes a sudden depolarization.

g. Critical Excitability Level of the Membrane Potential.—
Emphasis has already been placed upon the fact that if the de-
crease of the *Q* fraction produced by a cathodal current exceeds
a certain limit, the excitability of the nerve becomes depressed;
ultimately, the flow of the cathodal current renders the nerve
inexcitable. The effect of the cathodal current is not specific,
since it can be duplicated by any depolarizing agent. In general,
it can be said that a segment of nerve submitted to the action
of a depolarizing agent becomes inexcitable when the demarca-
tion potential measured between the treated segment and un-
treated nerve reaches the value of about 8–10 mv. The value of
the membrane potential at which the excitable mechanism be-
comes inoperative may be called the "critical excitability level."

The fact that the excitability of a depressed nerve can be in-
creased by an applied anodal current has been known for a long
time since it was discovered by Bilharz and Nase in 1862; in
addition, there is considerable literature on the restoring action
of the anodal current. In particular, it should be mentioned
here that in 1922 Thörner demonstrated that anodal polarization
may delay the onset of the inexcitability of a nerve deprived of
oxygen. It was not known, however, that the anodal current
may repolarize and render excitable nerves that have been de-
polarized far beyond the critical excitability level by the effect
of anoxia alone or combined with that of metabolic inhibitors.
In other words, it was not known that to a large extent an
applied anodal current may substitute for the activity of the
metabolic mechanisms of the nerve fibers.

The observations illustrated by figure 24 were begun after the
nerve had been deprived of oxygen for more than three hours.
Since the anoxic depolarization had decreased the membrane po-
tential below the critical excitability level the nerve was inexcit-
able. For this reason record *1* displays only the shock deflection,
i.e., the deflection produced by electrotonic spread of the stimu-
lating shock along the nerve. At the instant indicated by the
arrow on record *2,* a 12 μa anodal current was applied to the
nerve which was not interrupted until after record *11* had been

obtained. Since the nerve was in an advanced state of depolarization, the polarizability of the membrane was low; consequently the anodal current produced an increment of the membrane
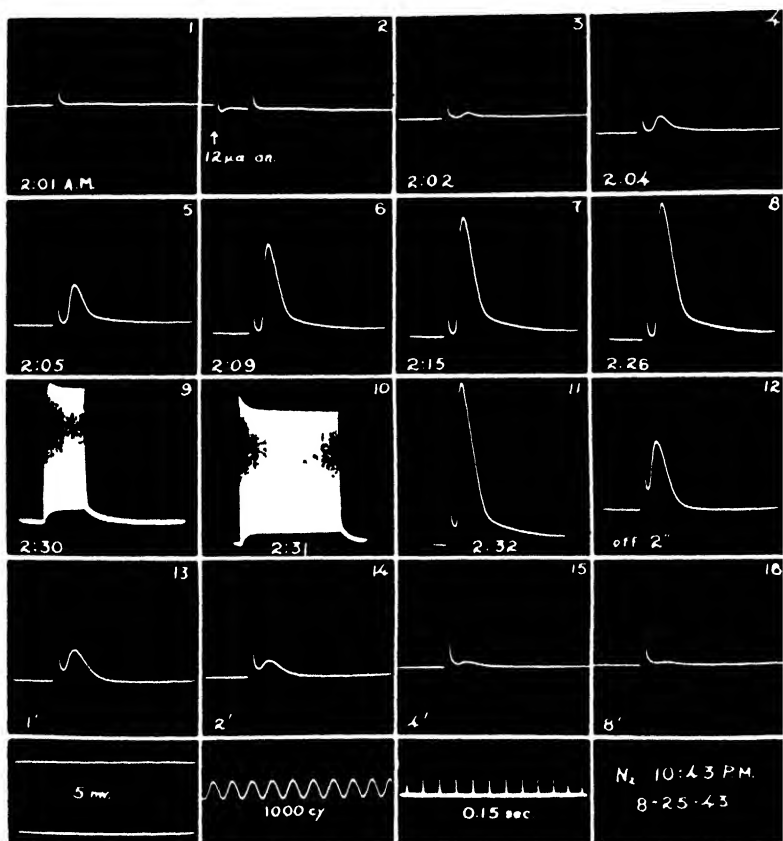

FIG. 24. Restoration of the excitability of anoxic nerve by an applied anodal current.

potential at a much slower rate than in the case of normal nerve. The increment of the membrane potential revealed itself in the form of a progressive displacement of the base line of records 2 to 11. Soon a few fibers of the nerve became excitable (record

3) ; the number of excitable fibers increased progressively (records *4* to *7*) until finally, after the anodal current had been allowed to flow for 25 minutes, a large majority of the nerve fibers were able to produce impulses (record *8*). The stimulating shock was then strengthened and many fibers were found to be able to produce impulses in response to a train of shocks at the frequency of 100 per second. The applied current was inter-
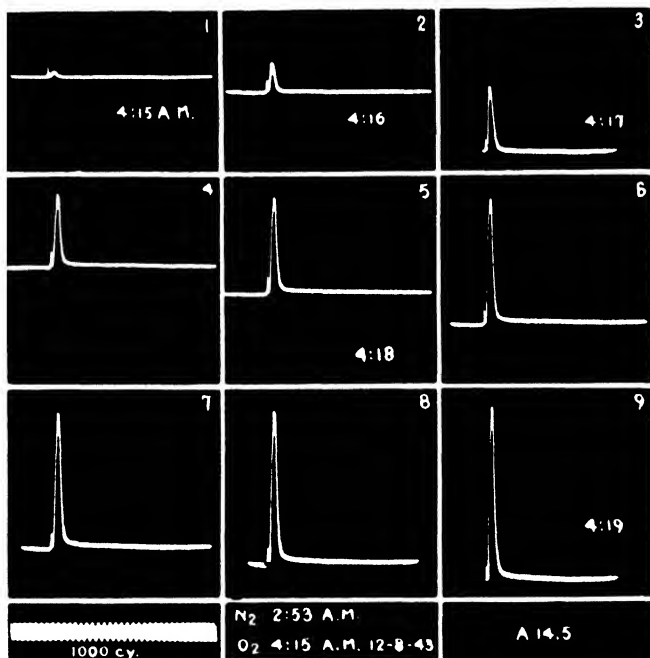
Fig. 25. Restoration of the excitability of anoxic nerve by respiration.

rupted immediately after the sweep of record *11*. In the absence of the applied current the nerve again became depolarized. The decrease of the membrane potential revealed itself in the progressive ascent of the base line of records *12* to *16*. As can readily be noted, the progressive depolarization was accompanied by a decrease of the number of excitable fibers, until finally the nerve again became inexcitable.

There is a remarkable similarity between the effects of anodal polarization and oxidative metabolism. In the experiment illustrated by figure 25 the nerve was deprived of oxygen until only a few fibers were excitable (record *1*). Oxygen was then introduced into the nerve chamber. Respiration resulted in a rapid increase of the membrane potential which was accompanied by restoration of the excitability of the nerve fibers.

In view of these results the following assumption seems to be justified. Nerve deprived of oxygen undergoes depolarization because in the absence of oxygen certain oxidized substances become reduced. Anoxic nerve is repolarized and rendered excitable by the anodal current because the flow of the anodal current results in oxidation of the reduced substances or, otherwise stated, because the flow of the anodal current produces changes in the physico-chemical structure of the nerve fiber similar to those which are produced by the uptake of atmospheric oxygen.

The results of experiments of the type illustrated by figure 26 suggest that indeed the anodal current produces its effect because the potential difference that it establishes across the membrane drives chemical reactions so that the changes underlying the polarization are reversed.

In the experiment illustrated by figure 26 the nerve was treated with iodoacetamide (0.001 M) and, after the symptoms of the poisoning had become patent, the nerve was deprived of oxygen. The observations recorded in figure 26 were made after the nerve had been deprived of oxygen for 180 minutes. Since the nerve was inexcitable, record *1* presents only the shock deflection. Anodal polarization was then applied throughout the intervals between records *2* to *17*. The magnitude of the current was increased and decreased several times in order to follow the progress of the restoration of excitability. During these trials it was observed that the number of excitable fibers was not directly dependent upon the value of the increment of the membrane potential; to be sure, there was at all stages of the restoration a value of the membrane potential at which the response was greatest, but restoration of the excitability of all the A fibers (record *15*)

was not obtained until the current had been allowed to flow for 11 minutes. This fact that can also be observed with unpoisoned nerve (fig. 24) clearly indicates that the restoration requires two
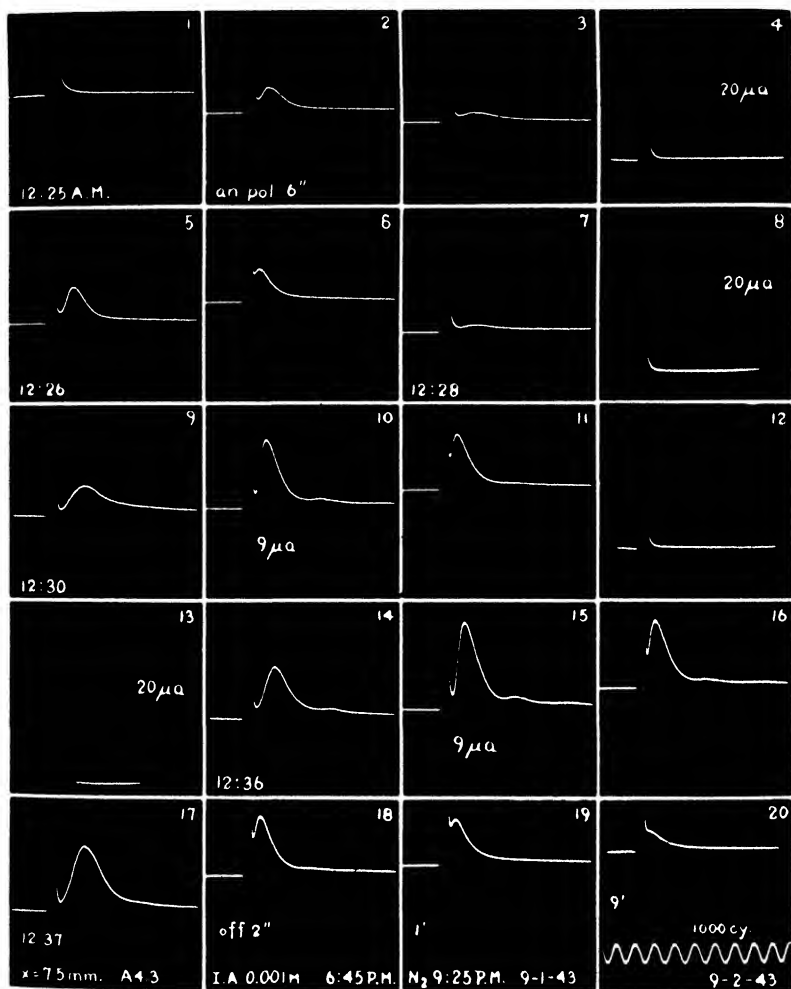

FIG. 26. Restoration of the excitability of nerve poisoned with iodoacetamide and deprived of oxygen, by an applied anodal current.

conditions: (1) that the membrane potential be raised above the critical excitability level, and (2) that the flow of current be maintained until chemical changes have taken place in the artificially repolarized membrane.

The extent by which the applied anodal current may substitute for the activity of the metabolic systems is illustrated by figure 27. It can be seen in figure 26, *18* to *20*, that after the interruption of the anodal current, the decrease of the membrane potential again rendered the nerve inexcitable. One hour later, the nerve having been maintained in nitrogen all the time, restoration of the excitability was again effected by means of the applied anodal current of optimal magnitude (9 μa). Record *1* of figure 27 presents the spike obtained in response to a maximal alpha plus beta shock. Without interrupting the flow of the applied current, the nerve was submitted to continuous tetanic stimulation that was maintained for two hours (records *2* to *24*). During the tetanus a progressive decrease of the value of the membrane potential took place (cf. base lines of records *2* to *5, 8, 9, 12, 13; 16, 17*) which was referable to the inability of the current to restore fully during the intervals between impulses the loss of membrane potential produced by each impulse. The loss of membrane potential resulted in the inexcitability of a number of fibers, but after the level of the membrane potential had been increased by means of a brief period of polarization with a greater current (records *6, 7; 10, 11; 14, 15; 18, 19*) the spikes regained practically their initial height (records *8, 12, 16, 20*). The applied current was interrupted immediately after the sweep of record *20;* the resulting depolarization of the membrane resulted in a rapid decrease of the excitability (records *21, 22*), but a 2-second period of polarization with a large current (record *23*) markedly increased the height of the response. Since the rhythmic stimulation of the nerve had been maintained for 127 minutes, the number of impulses which had been produced in the responding fibers may be estimated at no less than 150,000. Thus, there can be no doubt that in the absence of oxygen and in the presence of an inhibitor of glycolysis, the nerve fibers can produce an ex-

ceedingly large number of impulses provided only that their membrane potential be maintained at the appropriate level by means of an externally applied anodal current.
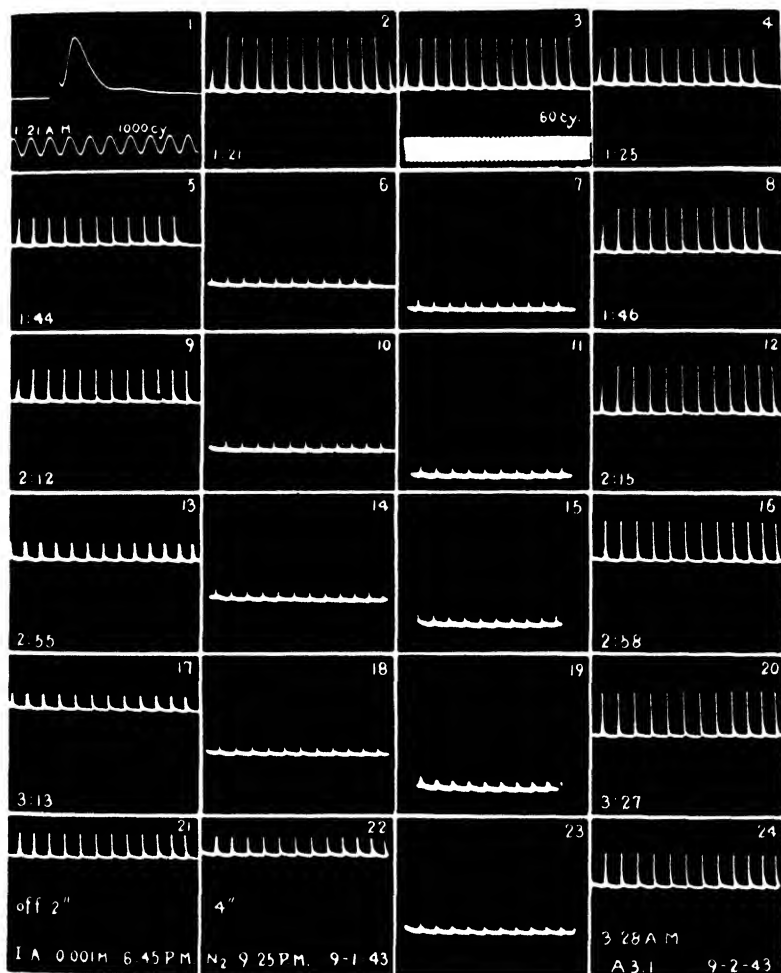

Fig. 27. Rhythmic activity of poisoned and anoxic nerve in the presence of applied anodal currents.

The significance of the experiment illustrated by figures 26 and 27 is increased by this fact. When oxygen was admitted into the chamber, the nerve failed to perform a successful oxidative repolarization; thus, there could be no doubt that the presence of iodoacetamide had blocked key reactions of the oxidative metabolism; nevertheless, anodal polarization still was able to effect restoration of excitability. Obviously, the anodal current may produce its effect even after important links of the chain of respiratory systems have been blocked.

The experiments illustrated by figures 26 to 27 were done with frog nerves; similar results, however, can be obtained with mammalian nerves. The observations presented in figure 28 were done with a human radial nerve that was dissected 13 hours after death. When the nerve was brought to the laboratory by Dr. Tarlov it was inexcitable; a small demarcation potential, however, was measured between the center of the nerve and a freshly injured point near one of the ends. The existence of this demarcation potential indicated that although the nerve had undergone a far reaching depolarization, a disintegration of its structure had not taken place yet; therefore, it was expected that if the nerve were artificially repolarized by an applied anodal current, it would regain its ability to produce impulses. The expectation proved to be correct.

Record *1* of figure 28 shows the response obtained after a several minutes long period of polarization with a 440 μa anodal current had begun to restore the excitability of the nerve fibers. The number of fibers which were able to produce impulses was exceedingly small; it increased, however, during the renewed flow of the applied current (records *2* to *4*). The break of the anodal pulse used to obtain records *5* to *6* initiated impulses in a number of fibers, some of which also were able to respond to the induction shock used to obtain record *7*. During a following period of anodal polarization (records *8* to *11*) the spike was seen to increase and after the end of the applied pulse the break response (record *11*) was greater than it had previously been (record *6*). A comparison of records *7* and *12* shows the increase of the num-

ber of fibers which had become able to respond in the absence of applied polarization.
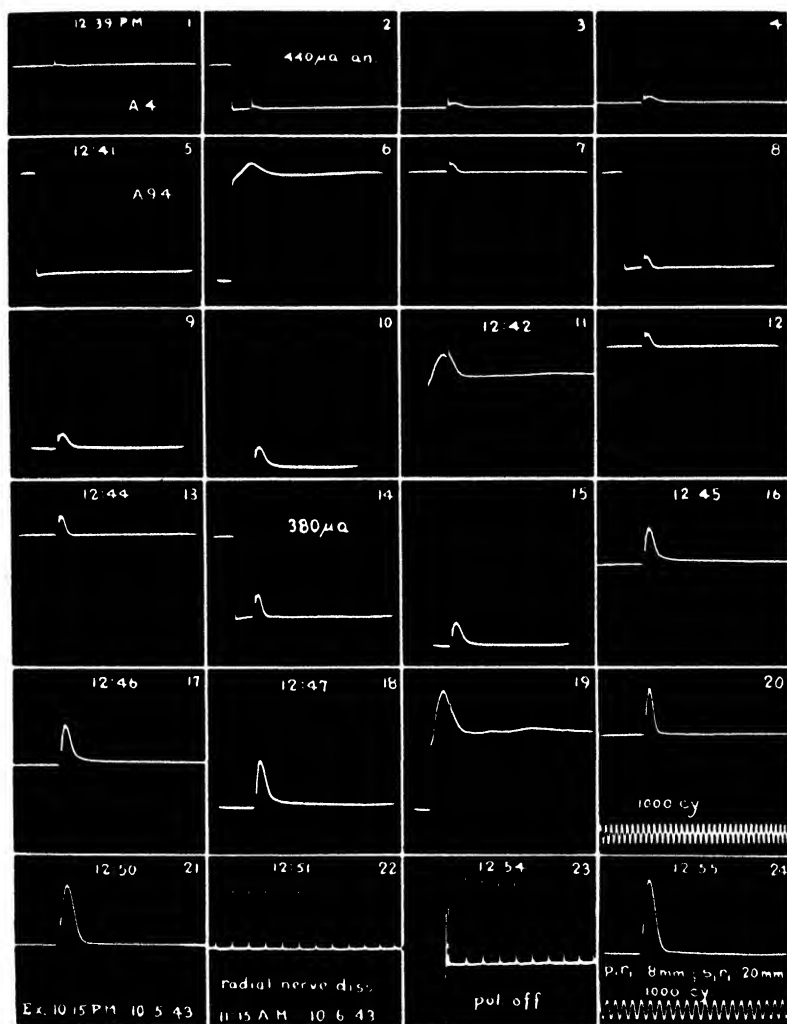

FIG. 28. Restoration of the excitability of a human nerve by the applied anodal current.

This number was further increased by a new period of polarization in the interval between the sweeps of records *12* and *13*. Since the state of the nerve was being rapidly improved, the magnitude of the applied current was decreased in order to avoid damage to the nerve. Restoration to an important degree was effected by the anodal pulse used to obtain records *14* to *19* and further improvement of the state of the nerve was produced by another pulse applied in the interval between the sweeps of records *20* and *21*. Records *22* and *23* present the responses to a rhythmic train of shocks at the frequency of 17 per second; anodal polarization was applied immediately after the sweep of record *22* and was interrupted during the sweep of record *23*.

One minute after the end of the polarization the spike produced in response to single shocks still had the great height shown by record *24*. In the absence of polarization the nerve fibers gradually became inexcitable; no difficulty was found, however, in restoring their excitability by anodal polarization. The shape of the spikes recorded in figure 28 as well as the results of appropriate tests done by displacing electrode r_1 away from the polarizing electrode (p_1), proved that the impulses were produced only in that segment of nerve which had been repolarized by the applied current.

h. *Concluding Remarks.*—Two general questions may now be considered. The first question is that of the relationship of the nerve impulse to the enzymatic systems of nerve. A complete answer to this question cannot be given at the present state of knowledge; a partial answer, however, is possible. Observations have been made on nerves that had been poisoned with a variety of metabolic inhibitors. Di-isopropyl fluorophosphate at the concentration 0.001 M or eserine up to the concentration 0.002 M do not prevent the conduction of impulses even after they have been allowed to act upon the nerve for 24 hours. Eserine at the exceedingly high concentration 0.01 M, fluoride at the concentration 0.02 M, cupric chloride at the concentrations 0.005 and 0.01 M, cyanide at the concentration 0.001 to 0.05 M and veratrine at the concentration 1 : 50000 cause a depolarization of the membrane

and consequently render the nerve fibers inexcitable. In all cases, however, anodal polarization is able to restore the excitability of the nerve fibers; therefore, the enzymatic reactions which are inhibited by those substances are not directly involved in the production of the nerve impulse. In point of fact, as the evidence stands at present, it is exceedingly unlikely that any of the enzymatic reactions which are now known could play a direct rôle in the production of the nerve impulse.

There can be hardly any doubt that the nerve impulse is the result of a reversible electro-chemical reaction, but the nature of this reaction still is a matter for conjecture. A successful approach to the problem could perhaps be made by studying the action of cocaine and similar anesthetics, since cocaine blocks the production of the nerve impulse selectively; that is to say, cocaine renders the nerve fibers inexcitable with little interference with other aspects of nerve function.

The second question is that of the relationship of the membrane potential to metabolism. There is no doubt now that the membrane potential is directly established by oxidative metabolism. It also can be taken for granted that applied currents modify the state and the properties of the membrane because by changing the value of the membrane potential they alter the course of reactions of the oxidative chain.

The effect of the cathodal current is opposed by the nerve reaction which tends to maintain the membrane potential at the normal level. If the nerve reaction is sufficient, that is to say, if the metabolic mechanisms are able to supply the energy required to maintain the strength of the double layers of the membrane at a constant level, the cathodal current fails to cause a progressively increasing depolarization of the nerve fibers. If the nerve reaction, however, is insufficient, that is to say, if the metabolic mechanisms are unable to replace the charged particles that are removed from the double layers by the current, the nerve fibers undergo a progressively increasing depolarization, the observed changes in the properties of the nerve fibers being exactly those which appear after the nerve is deprived of oxygen. On the other

hand, a nerve that has undergone anoxic depolarization is re-polarized by the anodal current much in the manner that it is repolarized by oxidative metabolism.

Since the value of the membrane potential determines the equilibrium point of a system of chemical reactions, the mem-brane potential must be regarded as a component of the system, the other components being, of course, the chemical species present in the membrane and core of the nerve fiber. The chemical con-stitution of the nerve fiber and the value of the membrane po-tential or rather of its fractions cannot be regarded as separate entities since a change in the chemical constitution must neces-sarily be accompanied by a change in the membrane potential and conversely, a change in the membrane potential must necessarily cause a change in the chemical constitution. Likewise, the mem-brane potential and the structure of the membrane must be re-garded as inseparable entities since the membrane potential de-termines the physico-chemical structure of the membrane and conversely.

Under conditions such as these it is clear that (1) a description of the properties of the membrane must always include a state-ment of the value of the membrane potential, and (2) a dis-tinction between "chemical" and "electrical" processes in nerve should not be made. To be sure, ordinary chemical reactions may take place in nerve but those reactions which are directly related to the maintenance of the resting membrane potential and to the production of the nerve impulse are electro-chemical reactions that take place at organized boundaries and can be driven in one direction or the other by a difference of electrostatic potential.

Only in one sense and solely for the purposes of theoretical analysis can a distinction be made between electrical and chemical processes. The resting membrane potential, i.e., the electrostatic potential difference that exists across the membrane is a measure of work done against Coulomb forces in separating charged particles of the opposite signs; consequently the membrane po-tential is the measure of an amount of free energy that has been released by degradation of certain chemical species. The existence

of a membrane potential indicates that free energy of metabolic substrates has been converted into electrical energy; according to the evidence which is now available this step is essential in the utilization of metabolic energy by the nerve fibers; it might also be important in the case of other cells.

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HYPERSENSITIVITY IN DISEASE
with Especial Reference to Periarteritis Nodosa, Rheumatic Fever, Disseminated Lupus Erythematosus and Rheumatoid Arthritis[1]

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NEARLY one hundred and fifty years ago, Edward Jenner (1), in the report of his studies on revaccination, recorded for the first time the observation that infection can alter the body in a manner that will cause its tissues to react with increased intensity to subsequent contact with the infective agent. Jenner's clearly recorded observations on this hypersensitivity caused by infection aroused so little attention that, when Robert Koch (2) rediscovered the phenomenon a century later during his studies on reinfection in tuberculosis, his observation was regarded as the first of its kind, and the intensified reaction to reinfection came to be known as the "Koch phenomenon."

Magendie (3) is, by tradition, usually credited with having been the first to have recorded the phenomenon of experimental anaphylactic shock, in 1839, but it was really not until about fifty years ago, following the introduction of diphtheria antitoxin, that clearly recognizable observations on experimental anaphylaxis began to appear; and it was not until the first decade of the present century that the pointed studies and writings of von Pirquet (4) on serum sickness, and of Wolff-Eisner (5) on hay fever, served to arouse a widespread interest in the anaphylactic state as a factor in human disease.

The past thirty years have witnessed a prodigious amount of investigation directed toward the understanding of the remarkable phenomena of hypersensitivity, and this effort on the part of many investigators has been rewarded by a notable increase

[1] Lecture delivered November 21, 1946.

in fundamental information, and by an astonishing revelation of important and altogether unsuspected rôles played by hypersensitivity in disease processes. It is, of course, quite impossible to deal with all of the important ramifications of this extensive and complex subject within the bounds of this lecture. I can best approach the subject from the paths that my colleagues and I have followed in our investigations; but in so doing I realize keenly that I shall have to pass over more hastily, or omit entirely, fundamental contributions to other aspects of the general subject, some of the most important of which have been made by members of this Society.

The substances capable of inducing the hypersensitive state are extremely numerous. They range from simple inorganic elements to organic products of microörganisms, plants and animals, and include, in addition, an ever increasing number of the products of industrial chemistry which enter into our daily lives. Opportunities for the development of hypersensitivity, therefore, surround us continually; and since the untoward manifestations of hypersensitive reactions are, at their least, uncomfortable and annoying, and at their worst, incapacitating or even fatal, it becomes obvious that, apart from the intrinsic fascination of the problems involved, an adequate understanding of the mechanisms and effects of the bodily alterations which convert so many relatively harmless substances in our environment into highly toxic agents, is a matter of no small moment from the standpoint of human welfare.

It is important, at the outset, to recall that there are two main types of sensitization, which differ from each other in a number of ways. One of these is the tuberculin type of hypersensitivity, so called because the reaction of the tuberculous body to the proteins of the tubercle bacillus is its prototype, though the same form of hypersensitivity develops as a result of infection with other bacteria, fungi and filterable viruses. The other is the anaphylactic type which, too, may result from the infection with microörganisms, but also from contact with a wide variety of substances derived from other sources. Each type arises from a

bodily alteration resulting from parenteral contact with a foreign antigen, or with a non-antigenic substance that is capable of uniting readily with body protein to form an antigen foreign to the host. When this bodily alteration has occurred, tissues will be injured, and even killed, by local contact with amounts of the foreign substance that would be harmless to the normal body; and constitutional symptoms, and even death will occur when amounts that are entirely harmless to the normal body enter the blood stream of the hypersensitive one.

In the case of the anaphylactic type of hypersensitivity we know that the essential alteration responsible for the sensitization is the production of an antibody that will react specifically with the foreign antigen. The presence of the antibody in the body fluids can be demonstrated by the precipitin test, or by injecting the serum of the sensitized body into a normal one, thereby conferring the sensitivity upon the latter; and a relationship between the amount of antibody and the degree of sensitivity has been established (6).

While as yet there is no definitive proof, there are very good reasons (7a), into which I cannot enter here, for believing that the tuberculin type of sensitivity is likewise dependent upon the presence of specific antibody, though in this case there is not a sufficient amount in the circulation to be demonstrated by passive transfer of the hypersensitivity.

There are additional and familiar differences between the two types of hypersensitivity. The introduction of the antigen locally into the tissues of the anaphylactically sensitized body incites a very prompt local inflammation which, except in severe reactions, is evanescent, whereas in the tuberculin type of hypersensitivity the local reaction develops slowly and progressively, and tends to persist for several days. The smooth muscles of the anaphylactically sensitized body are thrown into spasm on contact with antigen, but this does not occur in the case of tuberculin-type hypersensitivity. Still other differences exist (7b). Among them, and a striking difference, some years ago in studies carried out with Mrs. Lewis and with Dr. Follis we

found that while in both types of hypersensitivity extravascular tissue cells can be damaged and killed at the site of injection of the specific antigen, the death of the cells in anaphylactic local reactions is the result of vascular damage that interferes with the nutrition of the cells (8), whereas in the tuberculin type of hypersensitivity the cells of even an avascular tissue such as the cornea or, indeed, cells from the sensitized body, washed and isolated in tissue culture, are killed by contact with the specific antigen (8, 9). This demonstration of an intrinsic cellular sensitivity in the tuberculin type of hypersensitivity has been amply confirmed by others (10, 11, 12).

In contrast, it was found by Barg (13), by Aronson (14) and by ourselves that cells of the same types from the anaphylactically sensitized body are not damaged *in vitro* by contact with the specific antigen, even in the presence of antibody. Furthermore, it could be shown that, though antibody penetrates into the cornea, in the anaphylactically sensitized body the cells of the avascular cornea are not killed by contact with the antigen, even in a body so highly sensitized that the injection of a minute amount of the antigen elsewhere into a vascular tissue readily causes necrosis. If, however, in the sensitized animal a zone of the cornea were first vascularized, the application of the antigen produced there a violent inflammation, capillary thrombosis and hemorrhage, and the corneal cells in the immediate zone of vascular disturbance died (8).

It has been believed by some that the hypersensitive state responsible for asthma and hay fever in the human being is fundamentally different from anaphylactic hypersensitivity. I cannot enter here into a discussion of this point further than to say that I share the view of most present-day investigators that the apparent differences are overwhelmingly outweighed by the similarities, and are not of fundamental pathogenetic significance (7c). The local and systemic reactions produced by the injection of the specific antigen into patients with asthma or hay fever are, in all respects, typical anaphylactic reactions; and the pulmonary reaction and symptoms characteristic of asthma can

bodily alteration resulting from parenteral contact with a foreign antigen, or with a non-antigenic substance that is capable of uniting readily with body protein to form an antigen foreign to the host.   When this bodily alteration has occurred, tissues will be injured, and even killed, by local contact with amounts of the foreign substance that would be harmless to the normal body; and constitutional symptoms, and even death will occur when amounts that are entirely harmless to the normal body enter the blood stream of the hypersensitive one.

In the case of the anaphylactic type of hypersensitivity we know that the essential alteration responsible for the sensitization is the production of an antibody that will react specifically with the foreign antigen.   The presence of the antibody in the body fluids can be demonstrated by the precipitin test, or by injecting the serum of the sensitized body into a normal one, thereby conferring the sensitivity upon the latter; and a relationship between the amount of antibody and the degree of sensitivity has been established (6).

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readily be produced experimentally by causing the anaphylactically sensitized animal to inhale the specific antigen (15, 16).

The precise mechanism responsible for the characteristic reactions of the sensitized body to the antigen is not clear in either type of hypersensitivity. I shall not discuss this important and fundamental problem further than to recall, in relation to anaphylactic hypersensitivity, that histamine is capable of producing many of the anaphylactic phenomena in the normal body, and that in some (17) though not in all species (18), a substance with histamine-like activity is increased in the blood during anaphylactic reactions. Neither the site nor the manner of origin of that substance is definitely known.

While not all of the phenomena that occur during anaphylactic reactions are reproducible with histamine, there are no insuperable objections to the view that a histamine-like substance, liberated as a result of the antigen-antibody interaction, plays an important functional rôle in this form of hypersensitivity; and it would be as premature at present to discard this view, as some are willing to do, as it would be to accept it as conclusively established, as others are satisfied to do. That a substance with histamine-like activity can be liberated into the tissues locally is strongly suggested by the urticarial reactions produced in susceptible individuals by physical agents such as heat, cold, light and mechanical irritation (108). In some of these cases, notably in paroxysmal hemoglobinuria with urticaria, it has been demonstrated that the specific effect is mediated through an antibody-antigen reaction that takes place only under the influence of the physical agent, but in the majority of instances of ''physical allergy'' in which the attempt has been made to demonstrate an antibody, it has not met with success, and the physical agent, itself, appears to cause the liberation of an urticarial-producing substance.

The mechanism of the cellular damage in the tuberculin type of hypersensitivity is completely obscure, and there has been very little study of the matter. The peculiar, gradually evolving, or so-called ''delayed'' character of the reaction is similar

to that caused by streptococcal and diphtheria toxins and by certain chemicals, such as mustard gas.   It can be expected that *in vitro* studies of the effect of the specific antigen upon the metabolic enzyme systems of sensitized cells will greatly help to clarify the mechanism of the cellular damage.

The *tuberculin type* of hypersensitivity can play an important rôle in the pathogenesis of infection.   As a result of the development of this type of sensitization, even microörganisms which possess little toxicity for the normal body become able to damage seriously the tissues of the sensitized one.

In addition to local injurious effects upon the tissues, the absorption of the proteins of the microörganisms into the blood stream produces systemic symptoms; and after recovery from an infection that has produced hypersensitivity, the systemic symptoms characteristic of infection can readily be produced by the injection of a relatively minute amount of the bacterial protein, many multiples of which will cause no symptoms in the non-sensitized body.   In infections caused by microörganisms that possess little primary toxicity, there is good reason to believe that the symptoms which, in such infections, make their appearance only after a period of time sufficient for the development of hypersensitivity, are due largely to the action of the relatively non-toxic proteins upon the sensitized body.   In tuberculosis, for example, the injection of relatively large amounts of the bacillary proteins, or of large numbers of virulent tubercle bacilli, directly into the blood stream of the normal body produces no appreciable symptoms until hypersensitivity develops; but in the body that has been sensitized by infection or by vaccination with killed bacilli, the injection of far smaller numbers of bacilli, or of relatively minute amounts of the bacillary proteins, will cause severe constitutional symptoms and even death. Years ago, von Pirquet (4) recognized clearly the relationship between hypersensitivity and symptoms in various infections, and he suggested that the so-called incubation period in certain infections represents merely the asymptomatic period between the entry of the microörganisms and the development of hyper-

sensitivity to their products. While in certain infections the incubation period doubtless represents the period during which the microörganisms are proliferating to numbers sufficient to elaborate an appreciable amount of their own primarily toxic products, certainly in the case of microörganisms which have little primary toxicity, there exists persuasive evidence in support of von Pirquet's concept of the relationship between hypersensitivity and the incubation period.

The local and systemic injurious potentialities of hypersensitivity have long been thoroughly familiar to students of infection. In the early part of the present century, however, the idea that hypersensitivity is a protective mechanism in tuberculosis was advanced by Römer (19), and was subsequently freely transferred by others to infections in general (20, 106). The essence of that view was that since the tissues of the hypersensitive body are more readily damaged by the microörganisms, inflammation occurs at the site more promptly and markedly, and the inflammatory exudate inhibits mechanically the spread of the microörganisms from the site and brings about their more effective destruction. Thus, the two most important antibacterial manifestations of acquired immunity, i.e., the prompt prevention of dissemination of the bacteria and their more efficient destruction, came to be widely regarded as being dependent upon hypersensitive inflammation for their most successful operation, and in various infections hypersensitivity was believed to constitute the essential mechanism of acquired immunity.

This view had a wide appeal, and we, ourselves, had followed general opinion in accepting it until some years ago when, during studies on tuberculous infection carried out with Dr. McCordock (21), circumstances were encountered which we were unable to reconcile with the prevalent doctrine that hypersensitivity constituted the essential mechanism of acquired resistance in tuberculosis. An examination of the literature then revealed the remarkable fact that that doctrine had become accepted throughout the world, with few dissenting voices, although there had never been placed on record a single experiment or clinical

observation that demonstrated that hypersensitivity is necessary
for protection or for the more effective operation of acquired
resistance in tuberculosis or in any other infection, under any
condition whatever. The only thing actually known about the
effect of hypersensitivity at that time was that it rendered the
body more susceptible to injury by the infecting microörganisms
and their proteins. The rest was hypothesis. This had been
freely admitted by Römer (19), the originator of the hypothesis;
but his followers, without further evidence had become much
more dogmatic than he. It seemed, therefore, of more than theo-
retical importance to attempt to determine whether this state,
with its obvious injurious potentialities, is really necessary for
the effective operation of acquired resistance, and we engaged in
a study of this matter. Experiments of Mackenzie (107) had
indicated an independence of hypersensitivity and immunity, but
no distinction was made between anaphylactic and tuberculin-
type hypersensitivity. Observations by Swift and Derick (22)
had more strongly suggested that acquired immunity could exist
without tuberculin-type hypersensitivity, but the use of a non-
invasive bacterium of very low virulence in those experiments
had failed to provide an acceptable test of the matter. Using a
variety of highly virulent microörganisms and a variety of dif-
ferent experimental approaches, my colleagues and I were able
to show that there is no correlation between the degree of hyper-
sensitivity and the degree of acquired resistance; that hyper-
sensitive inflammation is incapable of preventing the early spread
of bacteria, or of protecting against infection, in the absence of
specific acquired resistance (28); that highly effective acquired
immunity can be established with the concomitant development
of hypersensitivity (23); that the immunity of an immunized,
hypersensitive animal can be passively transferred to a normal
animal without the concomitant transfer of the hypersensitivity
(24, 25); that acquired immunity remains intact after the aboli-
tion of hypersensitivity by desensitization (26, 27); in short,
that specific acquired immunity effectively inhibits the spread
of the bacteria and brings about their more efficient destruction

in the complete absence of hypersensitive inflammation.   Indeed, the studies demonstrated strikingly that the immune state, primarily through the action of antibody, enhances so greatly the protective power of the inflammatory mechanism that, far from requiring the exaggerated and accelerated inflammation of hypersensitivity, an astonishingly small amount of inflammation suffices for the effective protection of the body.   During the course of these investigations it was found that specific antibody is the agent responsible for the immediate prevention of spread of bacteria in the body with acquired immunity (25), and that antibody can exert this important function even in the complete absence of inflammation (29).

These various studies, which have been confirmed by many investigators in this country and abroad (7d), have demonstrated that acquired resistance is not dependent upon hypersensitive inflammation for its effective operation, and that when the capacity to react with hypersensitive inflammation is eliminated, the immunity mechanism operates unimpaired, and the tissues are spared from the damaging effects of hypersensitivity.

The bearing of these results upon the desirability of developing safe and efficient methods of preventing tuberculin-type hypersensitive reactions in infections in which they exert deleterious effects is obvious.

Let us now turn to a consideration of the *anaphylactic type* of hypersensitivity in the pathogenesis of disease.   In this type of hypersensitivity the known effects are those exerted upon vascular endothelium, upon smooth muscle and upon collagenous tissue.   Whether other effects, such as the hypersecretion of the bronchial and nasal glands, are due to a direct action upon the glandular cells, or to alterations in their nutrient capillaries, is not yet determined.   The significance of the tissue or blood eosinophilia that is so commonly associated with the anaphylactic reaction is still a complete mystery.

The anaphylactic injury to endothelium leads to increased permeability, and even to endothelial necrosis; smooth muscle is thrown into spasmodic contraction; affected collagen fibers

swell and undergo degeneration and fragmentation, and the interfibrillary substance becomes altered. These effects are responsible for numerous disease states in man. The spasmodic contraction of smooth muscle is familiar in the bronchial spasm of asthma, and in some of the gastrointestinal manifestations of hypersensitivity to foods. Anaphylactic vascular effects are familiar externally in eczema, urticaria, angioneurotic edema, purpura, and various forms of erythema. I wish here to dwell particularly upon the evidence that relates anaphylactic hypersensitivity to the internal lesions of a group of diseases that are highly important because of their incapacitating or lethal potentialities.

Recently, conclusive evidence has been obtained that the serious vascular disease, periarteritis nodosa, is a manifestation of anaphylactic hypersensitivity. The arterial lesion in this condition is characterized by edema, inflammation, degeneration of the collagen and, ultimately, necrosis of the cellular elements and loss of the elastic tissue. These alterations may lead to thrombosis of the vessel with infarction of the dependent tissue, or to aneurysm formation and rupture of the necrotic segment. The inflammatory cells are chiefly mononuclears and eosinophils. Though distributed widely throughout the arterial tree, the individual lesions are sharply focal, and commonly affect only a portion of the circumference of the vessel.

For many years the cause of periarteritis nodosa has been a matter of speculation and controversy. It has been attributed to a variety of microörganisms, to a filterable virus, to neurological influences and to toxic injury. Twenty years ago Gruber (30) suggested that it might be due to hypersensitivity. The presence of eosinophils was, itself, suggestive of that. Fifteen years ago Vaubel (31) found vascular lesions in animals that had been subjected to injections of foreign protein. He compared the lesions to those of periarteritis nodosa and of thromboangiitis obliterans, but more especially to what he termed "rheumatic arteritis." Miura (32) and Masugi and Sato (33) made similar observations.

Our own interest was first drawn to this problem as a result of the opportunity to study the tissues of a series of patients who died shortly after the onset of the systemic anaphylactic reaction typified by serum sickness.   Death from serum sickness, itself, rarely if ever occurs (Longcope (88)).   However, the use of sulfonamides in conjunction with foreign, antibacterial serum can serve to prolong life sufficiently to permit the development of serum sickness in patients who will eventually die of their infection in spite of the treatment.   Furthermore, shortly after the introduction of the sulfonamides in therapy it was pointed out by Hageman and Blake (34) and others that these drugs can produce a reaction altogether like that of serum sickness; and the studies of Schönholzer (35), Davis (36) and Wedum (37) have shown that the sulfonamides can attach themselves to plasma protein, and that the complex so formed can act as an antigen to sensitize the body anaphylactically to the sulfonamide radicle. The continued administration of a sulfonamide after a sulfonamide hypersensitive reaction has appeared can markedly intensify the reaction and cause a fatal outcome.   As a result of these unfortunate accidents, the widespread use of the sulfonamides greatly increased the opportunity to study the effects of the serum sickness type of anaphylactic reaction upon the tissues; and in a study of a series of patients who died shortly after the onset of this type of hypersensitive reaction, whether to foreign serum or to sulfonamides, we found at autopsy typical, fresh lesions of periarteritis nodosa (38, 39).

An examination of the scant literature on the pathology of serum sickness disclosed that Clark and Kaplan (40), in 1937, had noted similar vascular lesions in two patients who had developed serum sickness shortly before death.   The description of the lesions in their cases is excellent, but the authors hesitated to attribute the vascular injury to the hypersensitive reaction.   In the same year, Eason and Carpenter (109) observed widespread periarteritis nodosa in a patient who died nineteen days after the development of serum sickness, but they, too, were unwilling to attribute the periarteritis to the serum reaction.
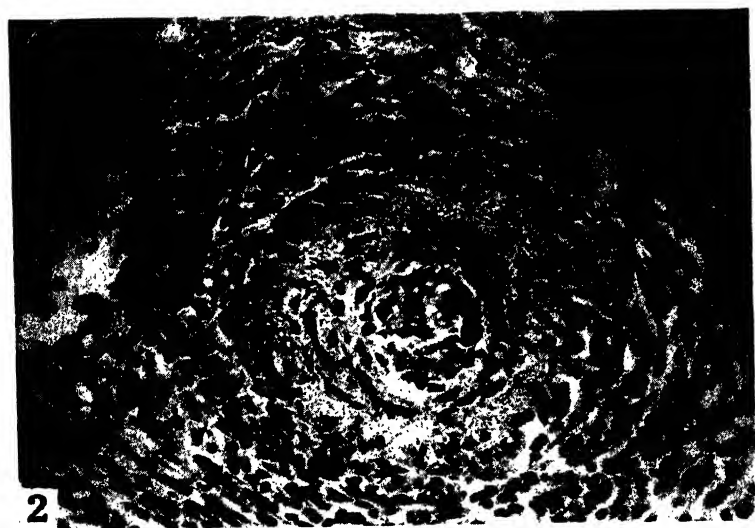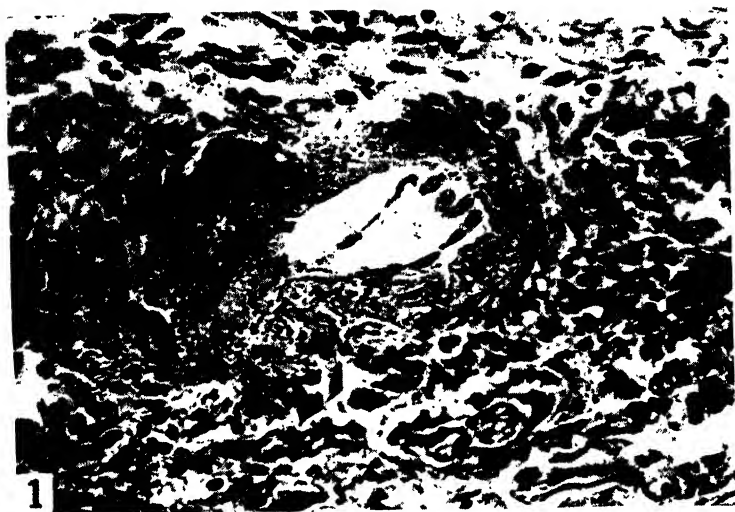
FIG. 1. Human periarteritis nodosa (Autopsy 17805).
FIG. 2. Experimental periarteritis nodosa in rabbit R8. See references 38, 42, 45 and 57 for illustrations of other stages of periarteritis nodosa in experimental and human anaphylactic reactions.

A careful analysis of our series of cases made it altogether improbable that the periarteritis nodosa was merely coincidental and unrelated to the hypersensitive reaction, and this led to an experimental study of the problem. Fleisher and Jones (41), some years previously, had shown that serum sickness can be produced in the rabbit by an appropriate intravenous administration of sterile horse serum. They described the clinical manifestations in a series of reports, but they made no study of the tissues. Dr. Gregory and I (42) used essentially the same method in the attempt to determine whether an uncomplicated anaphylactic reaction of the serum sickness type can produce periarteritis nodosa. Briefly, in studies that have been confirmed by Hopps and Wissler (110) and others, we found that widespread, typical lesions of periarteritis nodosa can be produced by this means (Figs. 1 and 2).

The demonstration that periarteritis nodosa can result from the anaphylactic type of hypersensitive reaction, indicates the advisability of searching for the sensitizing agent in all cases that come under clinical observation. Some instances may well be due to sensitization to foods, or to products of bacteria, of fungi or of parasites. Reimann has reported an association with trichinosis (87). It is pertinent that the figures of Rackemann and Greene (43) and of others (44, 111) show that periarteritis nodosa and asthma are associated with a frequency much greater than can be accounted for by mere coincidence. That sensitization to drugs other than the sulfonamides can cause this vascular lesion is now evident. We have described a case in which a patient with hyperthyroidism, under treatment with Lugol's solution, developed a hypersensitive reaction to iodine, characterized by fever, dermatitis, arthritis and eosinophilia. For certain reasons the treatment with iodine was continued. The course became stormy and the patient died. The autopsy showed widespread, fresh lesions of periarteritis nodosa (45). Friedberger and Ito (46) and Jacobs (47) have shown that a mixture of guinea pig serum and iodine will sensitize guinea pigs anaphylactically to the iodine radicle, presumably by reason of the

attachment of iodine to serum protein, thus forming a sensitizing complex.   Gibson and Quinlan (48) have lately reported a fatal case of periarteritis nodosa in a patient with hyperthyroidism who developed a hypersensitive reaction while under treatment with thiourea, a drug that is known to cause febrile, urticarial, anaphylactic reactions in man.   The sensitizing potentialities of certain drugs in common use by the laity, such as the phenolphthalein laxatives and aspirin, are familiar, and may well be kept in mind in this connection.   We have recently observed at autopsy

TABLE I

Cases of Periarteritis Nodosa
Department of Pathology, The Johns Hopkins Hospital

			Cases	
5 years	1916–1920,	1,902 autopsies	1	
5 years	1921–1925,	2,561 autopsies	1	13.6%
5 years	1926–1930,	2,774 autopsies	2	
5 years	1931–1935,	2,779 autopsies	2	
Sulfonamides introduced 1936				
5 years	1936–1940,	2,628 autopsies	15	86.4%
5 years	1941–1946,	2,579 autopsies	23	
			44	

a case of periarteritis nodosa in which the patient had volunteered the information that he had been taking aspirin, and that the drug had been causing a rash.*   While only some of those who become hypersensitive to a drug will develop periarteritis nodosa, it is clearly of no little importance to recognize hypersensitive reactions when they occur in patients under treatment with sensitizing drugs, and promptly to cease administration of the drug.   The

* Through the courtesy of Mr. J. J. Van Wyk, of The Johns Hopkins School of Medicine, I have recently had an opportunity to examine the tissues from a patient who died during a severe hypersensitive reaction to dilantin.   There were fresh lesions of periarteritis nodosa.   Dilantin is closely related chemically to nirvanol, the use of which had to be discontinued some years ago because of its marked sensitizing activity.

hypersensitive reaction is not always obvious in ill, febrile patients, particularly in negroes, in whom cutaneous rashes are more difficult to see; and this, doubtless, accounts for the fact that periarteritis from sulfonamide hypersensitivity continues to appear at autopsy (Table I).

While it is by no means certain that all of the effects of anaphylactic reactions upon the tissues are caused by histamine, a study of the influence of the recently developed anti-histaminic drugs upon the occurrence and progress of the lesions of periarteritis nodosa, of rheumatic fever and of the other diseases about to be discussed, is indicated.

In 1913, in a paper remarkable for the time at which it was written, Weintraud (49) formulated in the clearest possible manner the hypothesis that the lesions of rheumatic fever are caused not by a local action of microörganisms, but by an anaphylactic reaction to bacterial products liberated in a body sensitized by infection. During the past twenty years the possible action of hypersensitivity in the production of the lesions and symptoms of this disease has received much attention. The pioneering studies of Swift (50), followed by those of Coburn (51), in this country, and of Klinge (52) in Germany, have been particularly important in bringing this view into prominence. Against it, however, there has always been the lack of convincing evidence that lesions of the type characteristic of rheumatic fever can be caused by hypersensitivity. Several investigators attempted to produce lesions of the Aschoff body type by injecting bacteria into the tissues of sensitized animals. Gross (53) voiced general opinion in stating that the illustrated lesions did not really resemble Aschoff bodies, and he and others, repeating the experiments, were unable to confirm the claims. Klinge and his associates (31, 54, 98) described the occurrence of lesions, which they regarded as analogous to Aschoff bodies, in animals injected repeatedly with foreign protein. Their illustrations, likewise, were not convincing; and Bruun (55) was unable to produce lesions similar to the Aschoff body by that means, though he did observe lesions of different types which he believed

supported the concept that hypersensitivity plays a rôle in the pathogenesis of rheumatic fever.

In the hearts of some of the animals that were subjected to serum sickness in our experimental studies on periarteritis nodosa, we unexpectedly encountered lesions that were remarkably similar in their basic characteristics to those of rheumatic carditis. We therefore proceeded to study in further experiments the occurrence and nature of this anaphylactic carditis, which resulted from sensitization to foreign protein in the absence of infection or of bacterial products. In some experiments we used sterile horse serum as the agent to produce the serum sickness type of reaction; in others, egg albumin. From our experience, we think that Klinge and his associates probably observed in their experimental animals lesions that resembled rheumatic ones more closely than their illustrations would permit one to believe; or perhaps the method that we used may be more favorable for the development of lesions of this type than are other methods that have been used to elicit anaphylactic reactions.

As is well known, rheumatic carditis is characterized by multiple, focal lesions affecting all layers of the heart. Prominent are the alterations in the connective tissue collagen, the fibers of which, in sharply focal areas, become spread apart by edema, then swell markedly and finally degenerate, undergo fragmentation and may become fused with coagulated interfibrillary substance. The focal areas of collagen degeneration are ordinarily bordered by mononuclear cells, or the cells may be dispersed along the individual swollen fibers. A cardiac Aschoff body is merely a focus of degenerated collagen bordered by mononuclear cells, prominent among which are large basophilic cells with the spider-like distribution of chromatin characteristic of the "Anitschkow myocyte" that is peculiar to the connective tissue of the heart. In addition, there are focal areas of quite nonspecific inflammation in which fibrin, polymorphonuclears, lymphocytes, plasma cells and macrophages may be found. The valvular lesions, in their early stages consist in focal collagen

damage and focal inflammatory infiltrations. The endothelium may be damaged or destroyed, and minute, structureless vegetations may appear on the surface; or the endothelial cells may proliferate actively. Necrosis of minute foci of cardiac muscle, usually immediately adjacent to a focus of collagen degeneration, also occurs. Reparative fibrosis results in scarring of the valves and of all layers of the heart. In the hearts of our experimental animals we have observed lesions with all of these basic characteristics of rheumatic carditis, with the exception of the structureless surface vegetations upon the affected valves. These various lesions, several of which are reproduced here (Figs. 3, 4, 5, 6), have been abundantly illustrated in our papers (56, 57); and we have also produced in our experimental animals marked endarteritis of the coronary arteries, identical with that familiar in rheumatic fever (95).

Hopps and Wissler (110), repeating our experimental procedures, have recently confirmed the occurrence of periarteritis nodosa, myocarditis and lesions resembling Aschoff bodies in animals subjected to serum sickness. The valves were not studied histologically.[*]

We have pointed out in some detail elsewhere (56), and I think that it is important to repeat it here in summary, that not only have the lesions that characterize rheumatic fever in man the basic features of focal anaphylactic reactions, but that, in addition, a variety of associated anaphylactic manifestations occur during acute rheumatic fever. Thus, swelling and degeneration of collagen fibers has long been known to be a prominent feature of focal anaphylactic reactions (58, 59), and it is likewise a familiar characteristic of focal rheumatic lesions. Eosinophils, a hall-mark of anaphylactic reactions, are often present in large numbers in the acute lesions of rheumatic carditis, and blood eosinophilia has been observed repeatedly in rheumatic chorea

[*] Since this was written, Hawn and Janeway (Jour. Exp. Med., 1947, *85*, 571) and Crockett and Roberts (28th Ann. Session, Am. College Phys., May, 1947), also, have reported confirmation of the occurrence of periarteritis nodosa and cardiac lesions resembling those of rheumatic fever in rabbits sensitized by the intravenous injection of foreign serum.
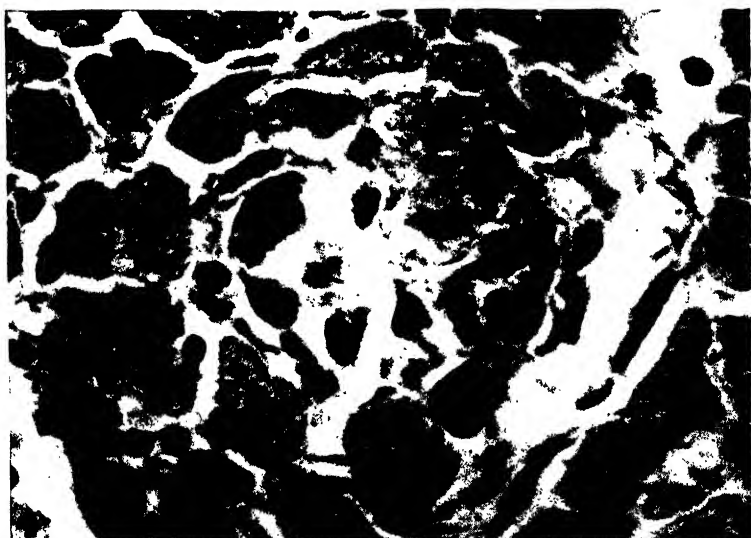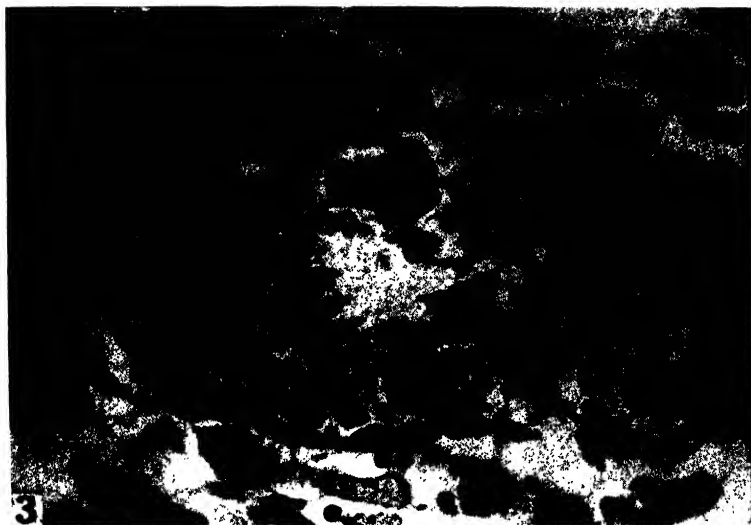
Fig. 3.   Acute rheumatic fever (Autopsy 18816).   Aschoff body in myo-cardium.
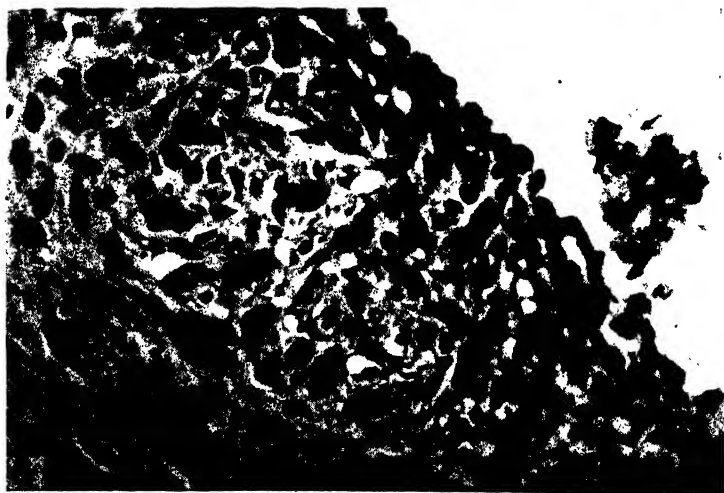Fig. 4.   Experimental Aschoff body in myocardium of rabbit R91PN6.
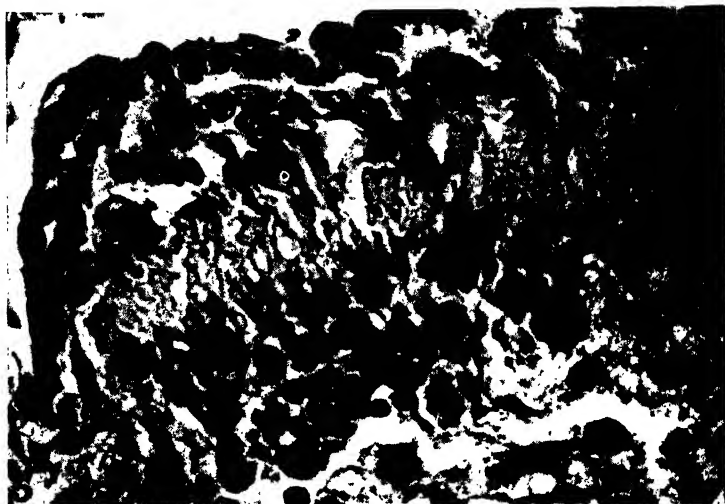
FIG. 5. Experimental rheumatic-like swelling and degeneration of collagen with marginal palisade of cells in endocardium of rabbit R48.

FIG. 6. Experimental rheumatic-like nodule in endocardium of rabbit R65PN3. Note the large, darkly-staining, multinuclear cells like those of the Aschoff body. ''Anitschkow myocytes'' are common in these experimental lesions.

(60, 61a).   It has been recognized for many years that inflammatory-necrotizing vascular lesions of the periarteritis nodosa type occur not uncommonly in the coronary arteries in rheumatic fever (62, 63, 79), and the relation of anaphylactic hypersensitivity to periarteritis nodosa is now established.   Purpura is a familiar manifestation of rheumatic fever, and it is definitely established that purpura can result from anaphylactic sensitization (64).   Other cutaneous lesions, such as urticaria, erythema multiforme and erythema marginatum, which occur in the anaphylactic reaction typified by serum sickness, are also familiar in rheumatic fever.   Indeed, rheumatic fever displays an impressive variety of phenomena that are now known to occur in the pure anaphylactic reaction of serum sickness.   In serum sickness, as in rheumatic fever, there occur anaphylactic cutaneous lesions, fever, arthritis that is relieved by salicylates (56), transient paresis (65), focal degeneration of collagen, tissue eosinophilia, vascular lesions of the periarteritis nodosa type, myocarditis and valvulitis (40, 57); and cardiac functional abnormalities, comparable to those occurring in acute rheumatic fever, have been observed in serum sickness (66, 67).

Another interesting rheumatic manifestation which has recently been identified as an anaphylactic phenomenon is migratory pneumonitis.   These sterile, pulmonary consolidations that occur in rheumatic fever are found histologically to represent focal areas of alveolar capillary damage with associated exudation, hemorrhage and capillary thrombosis, leading at times to necrosis of the affected alveolar wall (68, 69, 70).   Their anaphylactic nature is now apparent, for we have shown that this peculiar type of pulmonary lesion occurs not only in rheumatic fever but also in anaphylactic reactions to sulfonamides (70), and in association with anaphylactic periarteritis nodosa.   Furthermore, we have observed its basic characteristics in the lungs of animals killed during experimental serum sickness (71).

It is pertinent in relation to the histological evidence of the anaphylactic nature of these migratory pulmonary consolidations that Harkavy (72) and others (73, 74, 75) have observed their

occurrence clinically, in non-rheumatic patients, in association with bronchial asthma, with periarteritis nodosa, with urticaria and purpura, and with angioneurotic edema. Our studies have led us to regard these transient consolidations as the pulmonary analogues of cutaneous urticaria and purpura; and the recognition of their anaphylactic nature brings further evidence in support of the view that the lesions of rheumatic fever, wherever they occur, may represent alterations resulting from anaphylactic reactions to a foreign antigen.

A careful study of certain other important diseases, which exhibit clinically different manifestations, provides arresting evidence that they, too, may represent different combinations, localizations and degrees of anaphylactic collagen and vascular injury. Especially to be mentioned is disseminated lupus erythematosus, to the pathology and clinic of which Libman, Klemperer, Baehr and their associates have made notable contributions.

I need not dwell upon the well-known fact that, clinically, disseminated lupus has enough in common with periarteritis nodosa and with rheumatic fever to render the differential diagnosis difficult at times, and at certain stages impossible; but I do wish to point out and to demonstrate to you that in disseminated lupus, as in periarteritis nodosa and in rheumatic fever, there occur an impressive variety of lesions, all of which are familiar effects of focal anaphylactic reactions. In all three diseases there occur urticarial and erythematous cutaneous eruptions, purpura, arthritis, necrotizing-inflammatory arterial lesions, focal collagen degeneration, and myocarditis. Sterile inflammation of serous membranes occurs in all of them. Transient paresis, which may represent the effect of focal cerebral urticaria or purpuric hemorrhage, is common to them all.

In the cardiac lesions of rheumatic fever there is a tendency to more marked swelling and fusion of the affected collagen fibers than in disseminated lupus and periarteritis nodosa, and larger cells collect about these denser foci. This constitutes the difference between a typical Aschoff body and the focal cardiac lesions that occur in disseminated lupus and in periarteritis nodosa.

There are, however, many focal lesions in the rheumatic heart in which the degree of collagen swelling and the size of the associated cells are precisely like those encountered in disseminated lupus, periarteritis nodosa, serum sickness, and sulfonamide hypersensitivity (Figs. 11, 12, 13, 14, 19, 20) ; and, conversely, in these latter conditions there are occasional, though rare, lesions in which the state of the collagen and bordering cells is quite like that of an Aschoff body (Figs. 9, 10, 21, 22).

The verrucous valvular vegetations in disseminated lupus tend to be larger than those in rheumatic fever, but this, too, is a matter of degree, for the basic character of the vegetations is identical in both diseases (Figs. 7 and 8).   Indeed while rheumatic fever is universally regarded as the sole cause of mitral stenosis, the basic identity of the acute and subacute endocarditis in disseminated lupus leaves no doubt that the healing and scarring of those lesions would result in mitral stenosis; and it may well be that some of the cases of mitral stenosis, without other rheumatic stigmata, that are encountered at autopsy from time to time may represent instances of healed disseminated lupus.

The frequency of clinical pneumonitis of unidentified type in disseminated lupus has been stressed by Libman (76), and I can report that we have observed at autopsy in this disease the same anaphylactic type of pneumonitis that occurs in sulfonamide hypersensitivity, in periarteritis nodosa and in rheumatic fever.

Finally, I may say that we have found that the peculiar focal necrosis of the lymph nodes and spleen, which has been described as characteristic of disseminated lupus (99), occurs not only in lupus but also in periarteritis nodosa and in association with the anaphylactic type of reaction to the sulfonamides (38) and to iodine (45) (Figs. 15 and 16).   Often, if not always, this focal necrosis appears to result from arteriolar and capillary thrombosis.

Gross (77), from his study of the pathology of disseminated lupus, concluded that the primary and fundamental lesion in this disease is endothelial damage.   Klemperer and his co-workers (78) have taken issue with that, and regard collagen damage as

FIG. 7.   Rheumatic vegetation on mitral valve (Autopsy 18994).
FIG. 8.   Vegetation on mitral valve in disseminated lupus erythematosus
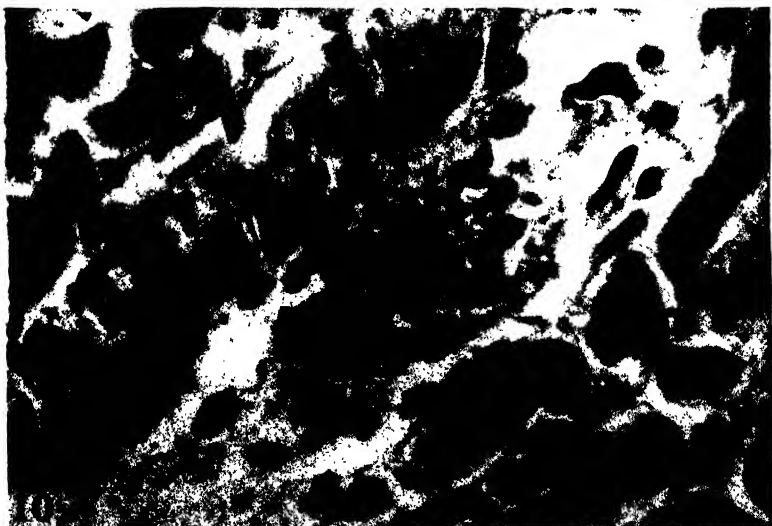(Autopsy 17994).   Compare with Fig. 7.

Fig. 9.   Acute rheumatic fever (Autopsy 17922).   Small Aschoff body in myocardium.

Fig. 10.   Disseminated lupus erythematosus (Autopsy 18285).   Small lesion of Aschoff body type in myocardium.   Compare with Fig. 9.   This was the only lesion of the Aschoff body type found in the sections of the heart.

FIG. 11. Acute rheumatic fever. Two zones of fibrinoid degeneration of swollen collagen fibers in epicardium.

FIG. 12. Disseminated lupus erythematosus (Autopsy 18285). Two zones of fibrinoid degeneration of swollen collagen fibers in epicardium, identical with those in Fig. 11.
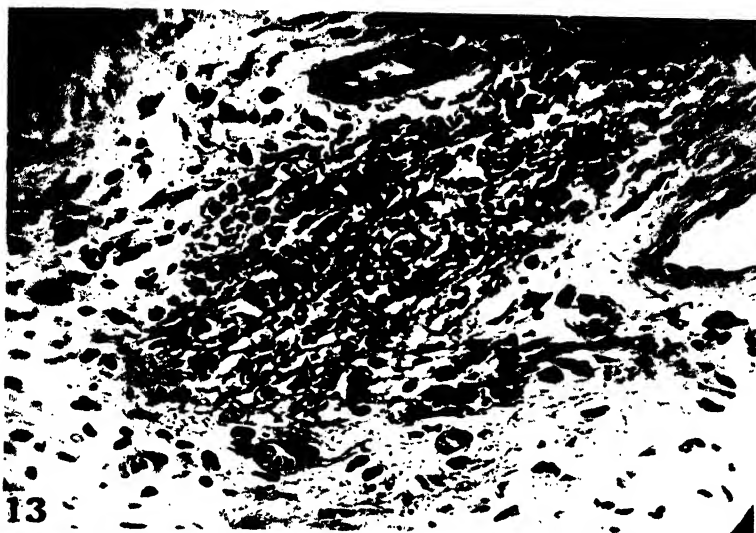
FIG. 13. Acute rheumatic fever (Autopsy 9104). Focus of swollen, degenerated collagen with associated cells in myocardium.

FIG. 14. Disseminated lupus erythematosus (Autopsy 17440). Focus of swollen, degenerated collagen with associated cells in myocardium.
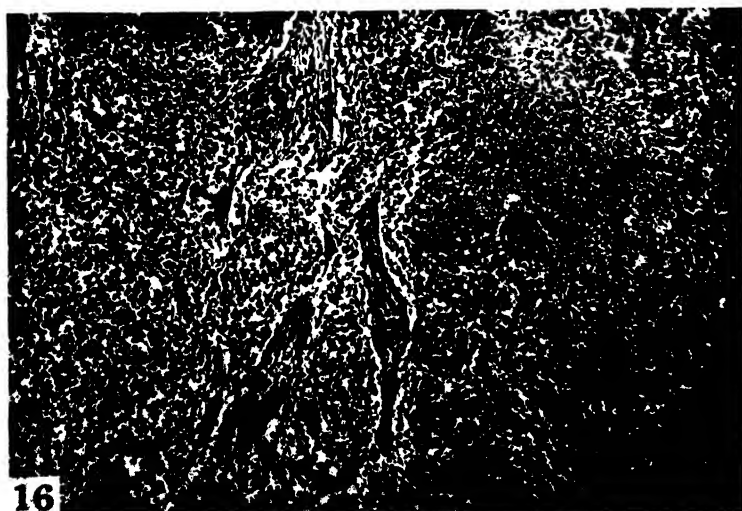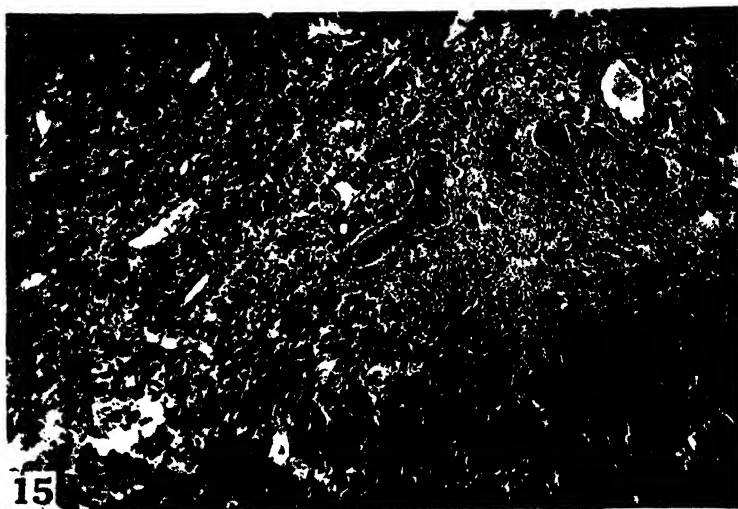
FIG. 15.   Disseminated lupus erythematosus (Autopsy 17051).   Necrosis (at right) in lymph node.

FIG. 16.   Human serum sickness type of anaphylactic reaction, with periarteritis nodosa, following foreign serum and sulfadiazine (Autopsy 17250).   Necrosis (at right) in lymph node.   Compare with Fig. 15.

the basic lesion.   The view that the lesions of disseminated lupus may be anaphylactic in origin obviates the need of these divergent interpretations, for both endothelial and collagen damage are characteristic effects of focal anaphylactic reactions.

Since it is commonly stated that disseminated lupus is "almost exclusively" a disease of females, it may be mentioned that over 20 per cent of the reported cases have occurred in males (112). Of 22 cases in the files of our department, 30 per cent have been males.

Among other conditions that deserve study from the standpoint of their relationship to the group of anaphylactic collagen-vascular diseases there must, of course, be mentioned Schönlein's purpura, with its associated arthritis, urticaria and other types of anaphylactic eruptions.   The anaphylactic nature of purpura with abdominal symptoms (so-called Henoch's purpura) has already been clearly demonstrated by Alexander and Eyermann (64).   There are increasingly strong indications that hypersensitivity, whether to a foreign antigen or to the body's own renal tissue through the mediation of an iso-antibody, is the pathogenetic basis of certain instances of glomerulonephritis. Any proper exposition of this important matter would require more time than remains at our disposal.   I do wish, however, to devote a few moments to a consideration of the relationship of rheumatoid arthritis to the group of diseases that we have had under discussion, and in particular to rheumatic fever.

The view that rheumatoid arthritis and rheumatic fever may be different manifestations of one basic process is an old one, and has been reviewed and supported by Dawson (90) and others; but the clinical differences have led most physicians to regard the two conditions as quite separate diseases.   In rheumatic fever, in which the attack on the joints is acute, transient and infrequent, deforming arthritis is correspondingly rare.   It is well known, however, that there do occur instances in which the arthritis in clinically typical rheumatic fever is persistent, and progresses to the condition typical of rheumatoid arthritis. Indeed, the difference between the joint manifestations in rheu-

matic fever and in rheumatoid arthritis might well be no more than the difference between the effect of infrequent and rapidly subsiding attacks upon the joint and a chronic, smouldering process of the same basic type.

Sterile pericarditis occurs in rheumatoid arthritis, as it does in rheumatic fever, periarteritis nodosa and disseminated lupus; but the infrequency of clinical involvement of the myocardium and valves in rheumatoid arthritis has always been advanced as a strong reason for regarding this condition as basically different from rheumatic fever. Since there is now abundant proof that even the same antigen may produce its anaphylactic effects upon selectively different tissues in different individuals, an anaphylactic involvement of the joints without myocardial involvement need be no more anomalous than the fact that the ingestion of milk can cause an attack of asthma in one hypersensitive individual, urticaria in another, and both in a third. As a matter of fact, however, recent studies may necessitate a revision of the widespread belief that the heart is rarely involved in rheumatoid arthritis.

Until recently there has been surprisingly little careful pathological study of the heart in groups of cases of rheumatoid arthritis. The evidence provided by investigations during the last five years indicates that cardiac lesions may occur in this disease more frequently than clinical observation would lead one to expect. In 1941, Bagenstoss and Rosenberg (80) found cardiac lesions, which they described as "identical with those of rheumatic fever," in 56 per cent of 25 autopsies on patients with clinically typical rheumatoid arthritis. In one third of the cases typical Aschoff bodies were present. Bayles (81), in 1943, reported that of 23 cases of typical rheumatoid arthritis studied at autopsy "22 per cent had rheumatic cardiac lesions." In 1944, Young and Schwedel (82) found what they regarded as "definite rheumatic" cardiac lesions in 65 per cent of 38 cases of typical rheumatoid arthritis.

Bagenstoss and Rosenberg (80) were inclined to attribute the discrepancy between the clinical and the pathological evidence

of cardiac involvement in their cases of rheumatoid arthritis to a less extensive distribution of the cardiac lesions than in rheumatic fever, though the actual character of the lesions was the same. Further pathological studies of the heart in rheumatoid arthritis are urgently needed; and since there may be differences of opinion as to what constitutes a typical rheumatic lesion, the cardiac lesions encountered in each case of rheumatoid arthritis should be clearly illustrated.

Focal collagen degeneration of the anaphylactic type is well known to occur in rheumatoid arthritis. Indeed, the familiar rheumatoid subcutaneous nodule is, in essence, a reaction to a focus of degenerated collagen, and is basically identical with the subcutaneous nodule of rheumatic fever, as Dawson (96), Klinge (52) and others have pointed out. Our own studies place us in entire agreement with those who regard various suggested differences (91, 97) between the rheumatic and the rheumatoid nodule as being no more than differences in the intensity and chronicity of an identical process in different individuals (Figs. 17 and 18). It is of interest that we have observed the same basic type of lesion, on a smaller scale, in periarteritis nodosa (Figs. 21 and 22); and, of course, the Aschoff body of rheumatic fever is, itself, only a miniature of the subcutaneous nodule, i.e., a tiny focus of degenerated collagen bordered by mononuclear cells. It is hoped that appropriate studies will soon provide much-needed information regarding the precise mechanism that produces the focal collagen degeneration in these various conditions.

While, in the early stages, it may be difficult or impossible to differentiate rheumatoid arthritis from rheumatic fever, a well-developed case of chronic rheumatoid arthritis is, of course, quite different clinically from one of rheumatic fever. Clinical signs and symptoms are, however, hazardous criteria of etiology or pathogenesis. A case of chronic pulmonary tuberculosis can be very different clinically from one of acute miliary tuberculosis, though the same etiological agent is responsible for both; and a case of angioneurotic edema is very different clinically from one of asthma, though the pathogenetic basis of both is anaphylactic
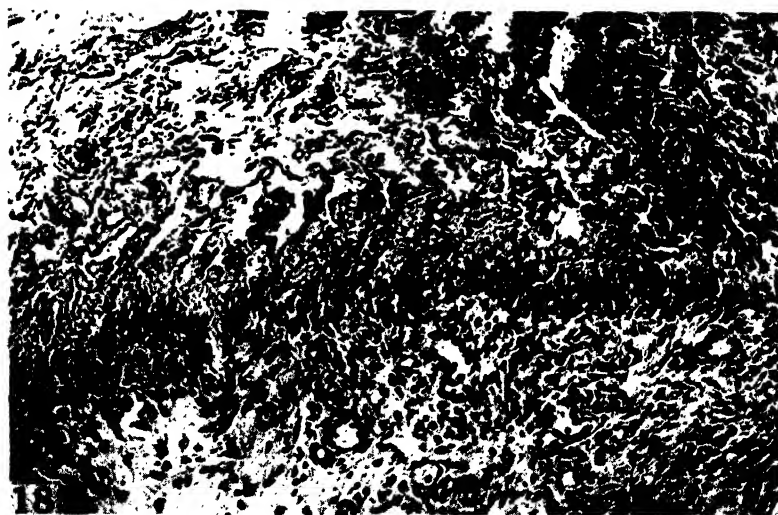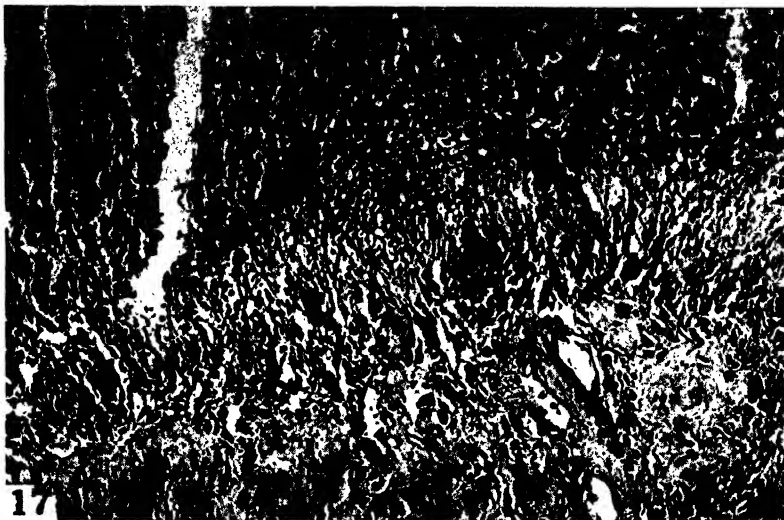
Fig. 17.   Rheumatoid arthritis (S.P. Col. 285).   Subcutaneous nodule.
Note swollen, degenerated and fused collagen with marginal palisade of
cells.

Fig. 18.   Acute rheumatic fever (S.P. 91381).   Subcutaneous nodule
basically identical with rheumatoid nodule in Fig. 17.
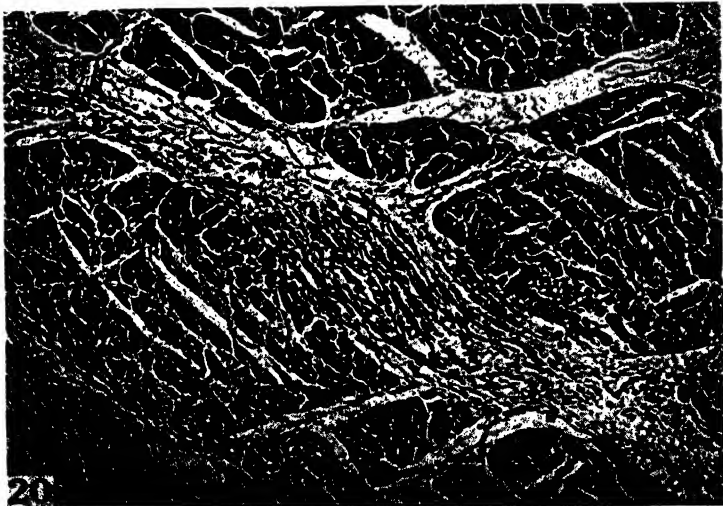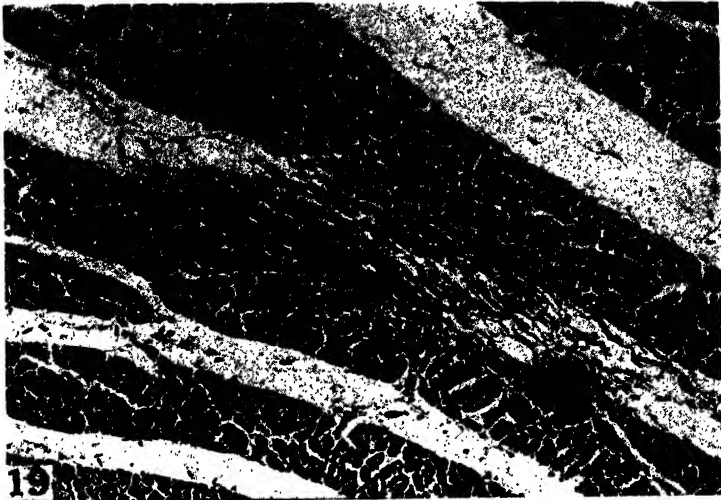
FIG. 19.   Acute rheumatic fever (Autopsy 17922).   Focus of swollen, degenerated collagen with associated cells, in interstitial tissue of myocardium.

FIG. 20.   Generalized periarteritis nodosa (Autopsy 17805).   Focus of swollen, degenerated collagen with associated cells, in interstitial tissue of myocardium.   Compare with Fig. 19.

FIG. 21. Acute rheumatic fever (Autopsy 8981). A zone of degenerated, fused collagen with surrounding cells of Aschoff body type in myocardium.

FIG. 22. Generalized periarteritis nodosa (Autopsy 17805). A zone of degenerated, fused collagen surrounded by mononuclear cells of same character as those in Fig. 21. *This lesion is in a renal pelvis.*
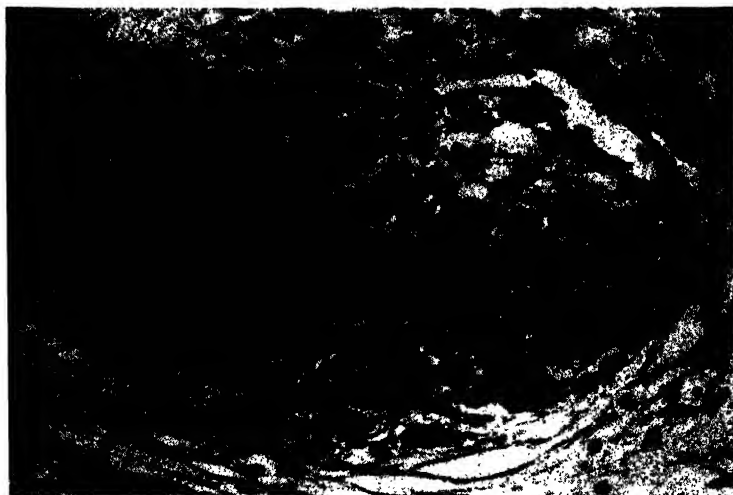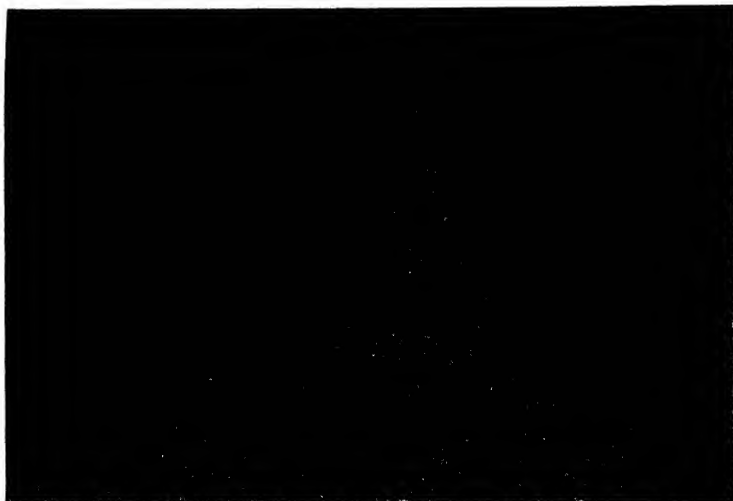
hypersensitivity.   On the other hand, it is equally important to emphasize that basic identity of lesions is not a proof of identity of etiology.   An abscess, for example, may have as its cause any one of numerous different types of bacteria, and vascular necrosis and deterioration of collagen can be caused by injuries other than anaphylactic reactions.   I should like to make it very clear, therefore, that while it is now established that periarteritis nodosa can result from protracted anaphylactic reactions, both in experimental animals and in man, we are not at liberty to conclude that necrotizing vascular lesions can be caused only by hypersensitivity; nor can we yet draw final conclusions regarding the role of hypersensitivity in the pathogenesis of the other diseases under present consideration, even though the lesions of these diseases are of the type that anaphylactic hypersensitivity can produce. However, in all of these diseases, not only are the basic tissue disturbances those that are known to occur in anaphylactic reactions, but these diseases also exhibit in common a wide variety of manifestations which clinical, pathological and experimental studies have shown to be effects that the anaphylactic state can produce (Table II).

It should be borne clearly in mind that in the case of lesions in which hypersensitivity constitutes the actual *pathogenetic* mechanism of injury, the *etiological* agent may be quite different in different cases.   Certainly, in the case of periarteritis nodosa it is now clear that the anaphylactic vascular lesions can be caused by sensitization to substances as widely different in character as foreign proteins, iodine and the sulfonamides.   In the case of rheumatic fever, there is persuasive evidence from the studies of Coburn (51) and others that the hemolytic streptococci are particularly active etiological agents, and Dr. Berthrong, Dr. Smolens and I are at present engaged in a study of the activity of the different sensitizing antigens of these bacteria.   However, streptococcal infection cannot be demonstrated in all cases of rheumatic fever, and our experimental studies indicate that lesions of the rheumatic type may be produced by sensitivity to other antigens as well.   That the hemolytic streptococci are more

TABLE II

	Experimental serum sickness	Human serum sickness	Periarteritis nodosa	Rheumatic fever	Dissem. lupus erythematosus	Sulfonamide hypersensitivity	Iodine hypersensitivity	Rheumatoid arthritis
Fever	++	++	++	++	++	++	++	++
Arthritis arthralgia	++	++	++	++	++	++	+	++
Urticaria	++	++	++	++	+	++	++	+
Erythemas	++	++	++	++	++	++	++	0
Purpura	++	++	++	++	++	++	++	+
Transient paresis	❢	++	+	++	++	❢	+	0
Tissue or blood eosinophilia	++	++	++	++	0	++	++	0
Focal collagen degeneration	++	++	++	++	++	++	++	++
Lesions of Aschoff body character	++	❢	+	++	+	+	❢	+
Endocarditis valvulitis	++	++	+	++	++	+	❢	+
Myocarditis	++	++	++	++	++	++	++	+
Sterile pericarditis	++	❢	++	++	++	+	❢	++
Sterile pleuritis or peritonitis	+	❢	+	++	++	0	❢	0
Anaphylactic-type pneumonitis	++	❢	++	++	++	++	❢	0
Necrosis and inflammation of arteries	++	++	++	++	++	++	++	+
Necrosis of spleen and lymph nodes	❢	❢	++	++	++	++	++	0
Significant glomerular capillary damage	+	++	++	0	++	+	❢	0

++ = Observed association.
+ = Occurs, but infrequent or slight.
0 = No apparent association.
❢ = Insufficient study.

effective than other bacteria in producing the natural disease may be simply an indication that these streptococci contain a substance that is particularly active in producing hypersensitive reactions of this type, just as certain foods, such as egg white, strawberries and shell fish, are much more active in causing urticarial anaphylactic lesions than are many other food-stuffs.

In this connection, it is important, in the case of each complex sensitizing agent such as a bacterium or a food-stuff, to learn whether its damaging sensitizing activity is due to a native, complete antigen, or to a non-antigenic component that is chemically suited to ready linkage with the host's body protein, with the resultant formation of an active sensitizing antigen, in accordance with Landsteiner's fundamental studies, and as we have seen to be the case in hypersensitivity to non-protein chemicals such as the sulfonamides and iodine. It is also highly important to learn whether certain lesions affecting specific tissues may be due to auto-sensitization, i.e., sensitization to specific substances present in the host's own tissues. The concept that under various influences, such as contact with bacterial products, a specific substance in a given body tissue may become altered in a manner that renders it capable of inciting the formation of a sensitizing antibody specific for that tissue and capable of injuring that tissue, is an old one (83), and it has received suggestive support from studies such as those of Elschnig (94) and Woods (84) on sympathetic ophthalmia, and of Schilling-Siengalewicz and Bieloszabski (85), Hertle and Pfeiffer (86) and others on homologous tissue antibodies. Paroxysmal hemoglobinuria, with its associated urticaria (61b), is, of course, well known to be caused by an auto-antibody. Schwentker and Rivers (100) produced antibody to brain tissue by the injection of autolyzed homologous brain, and Morgan (101) and Freund (89) have recently shown that suitable injections of homologous brain tissue will cause sensitization to brain tissue and encephalomyelitis. Schwentker and Comploier (92) were able to produce antibody to rabbit kidney by injecting into rabbits an emulsion of rabbit kidney mixed with streptococcal toxin; and Cavelti (93) has re-

ported that iso-antibody for renal tissue produced by this means acts upon the animal's own kidneys to cause nephritis—a matter which, if confirmed, would have obvious and far-reaching implications. The entire question of tissue-specific auto-antibodies is one that demands intensive exploration in relation to the diseases under present consideration.

Finally, I must mention very briefly two fundamentally important, though at present inexplicable, circumstances relating to anaphylactic hypersensitivity. First, there is the well-known fact that different individuals who become hypersensitive to a given antigen differ markedly in their tendency to develop damaging reactions on contact with the antigen. Similarly, only some of a group of animals sensitized to a given protein will develop periarteritis nodosa or cardiac lesions on contact with that protein. Secondly, there is the equally well-known fact that a given antigen will exert its effects on different tissues and in different ways in different individuals sensitized to it. Thus, different individuals sensitized to the same antigen may develop on contact with the antigen such different and separate reactions as urticaria, asthma, purpura, eczema, a gastrointestinal upset, vasomotor rhinitis, pneumonitis or arthritis. There is much evidence that, in man, the susceptibility to develop hypersensitive reactions such as hay fever and asthma is governed, in part at least, by heredity; and the studies of Wilson (102) and of Paul (103) indicate that heredity plays a rôle in susceptibility to rheumatic fever as well. In the experimental animal the influence of heredity on the development of hypersensitive reactions has been demonstrated by inbreeding (104, 105). However, the actual mechanisms that govern the differences in susceptibility to hypersensitive reactions in different individuals, and that determine which tissue, and what sites in that tissue, will be affected in a given individual, are still obscure. One need not labor the importance of striving to obtain a better understanding of these basic matters.

The studies on the tuberculin type of hypersensitivity that have been reviewed here have served to free us from the fatalistic doctrine that hypersensitive tissue damage, when it occurs, is a

sacrifice that has to be endured in order to obtain protection against infection; and the search for safer and more effective procedures directed towards abolishing this type of hypersensitive reaction in infections in which it exerts damaging effects becomes as rational as similar efforts directed against the anaphylactic type of hypersensitivity in asthma and in hay fever.

In relation to anaphylactic hypersensitivity, the reviewed studies provide evidence that focal collagen and vascular damage, of basically the same types that characterize the lesions of an important group of diseases, can result from focal hypersensitive reactions arising from the presence of a sensitizing antigen circulating in the fluids of the anaphylactically sensitized body. These studies have served to establish the rôle of hypersensitivity in the pathogenesis of periarteritis nodosa, and they point the way to paths of investigation that can lead to a better understanding of the pathogenesis of the other diseases of this group, in which there is conspicuous collagen and vascular injury. Numerous observers have been impressed by the clinical and pathological overlapping and transitions between these various conditions, and have suggested that some common denominator exists between them. I have endeavored here to present some of the evidence that indicates that anaphylactic hypersensitivity may be that common denominator. It is hardly necessary to emphasize the need of intensive investigation at the bedside and in the laboratory in order to determine the etiological agent in each particular case, and to define the rôle of sensitization in the pathogenesis of each particular type of lesion in which the influence of hypersensitivity is strongly indicated by the reviewed evidence, but not yet conclusively established. From the standpoint of the entire field of hypersensitivity, there is high promise in an attack on the virtually untouched, fundamental problems relating to the intimate chemical mechanisms through which hypersensitive reactions exert their injurious effects upon the tissues. Apart from their very great intrinsic interest, studies directed properly towards these ends can hardly fail to be productive of important practical consequences.

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THE PROSTATIC SECRETION[1]

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THIS paper is concerned with some simple experiments in the physiology and biochemistry of the prostatic secretion— both the process and the product. We hope that the results have contributed to a better insight into the workings of nature and have added a little to the comfort, if not the edification, of mankind. The motivating premise in this work was that the *systematic* study of pathologic states is possible only in proportion to what is known about the activity of the normal tissues. Concerning the simplicity of an experimental approach, bear in mind that one of the aims of science is to obtain unequivocal results and if the unknown may be resolved by easy methods it is preferable. The experimental techniques to be presented are unique in that they reveal in a connected and quantitative way the activity of the male genital complex over many days.

The secondary sex characteristics develop from stimulation of target areas by the sex hormones and are of two types—self-sustaining or dependent. There are certain structures such as the larynx and beard of the human male which require androgen for their development to the adult state but having been so constituted they are quite capable of sustaining themselves in an essentially mature form on subsequent androgen deprivation. Then again there are other tissues such as the comb of the chicken and the normal mammalian prostate which cannot maintain the permanent survival values of maturity independent of androgen but shrink when the growth stimulus is taken away.

The prostatic secretion is an end product with an involved antecedent background which is now becoming well understood.

Since the adult prostate is not self-sustaining its secretion can be easily adapted to serve as a quantitative indicator of the activity of many hormones especially as they affect androgen.

The natural production of androgen results from an intricate interplay of chemical forces in which the protein and steroid hormones of pituitary and testis are very important; prostatic secretion in normal animals indicates that this system and also the receptor organ are reasonably intact. If any link in this endocrine chain is broken or rusty it may be replaced or reinforced for experimental purposes by substitution measures, of which a wide variety of effective agents is now available, especially the steroids and gonadotrophins.

VOLUMETRIC STUDIES OF SECRETION

Prostatic Isolation Operation. Eckhard (1) in 1863 ligated the neck of the bladder in dogs and placed a cannula in the urethra to deliver prostatic secretion which he obtained in copious amount after electrical stimulation of the *nervus erigens,* an anatomical structure that he had just discovered. Several studies of this type were then carried out (2, 3) and an ingenious technique was devised by Farrell (4) but because of technical difficulties only observations in "acute" experiments were reported by these workers. In order to study prostatic secretion for longer periods, a simple technique (5) of prostatic isolation (Fig. 1) was devised in 1939; in dogs the bladder was separated from the prostate, the urine was deviated through a brass inlaying suprapubic cannula and circumcision was done. Healing was complete in about seven days and the ensemble permitted subsequent assay of the prostatic secretion at frequent intervals for more than two years in some dogs; the animals remained in good health, thriving and gaining weight. The dog is a convenient species for this testing since it tolerates the experimental procedures well; moreover, it does not have seminal vesicles and the prostatic secretion (5) amounts to more than 97% of the semen.

Normal Prostatic Secretion of Dogs. The prostate gland of adult dogs secretes small quantities of fluid, about 0.1 to 2 cc.

per hour without external stimulation; dogs with the isolation operation continue to be attracted by vertical structures which they approach at frequent intervals to deliver prostatic fluid from the urethra. The secretion is greatly augmented by cholinergic agents (2). For adult dogs, pilocarpine hydrochloride, 6 mg. intravenously, is an appropriate stimulus although this amount does not give maximal secretion; actually the secretory output of the prostate increases nearly logarithmically until a dosage is reached which produces fatal systemic effects; dog 136
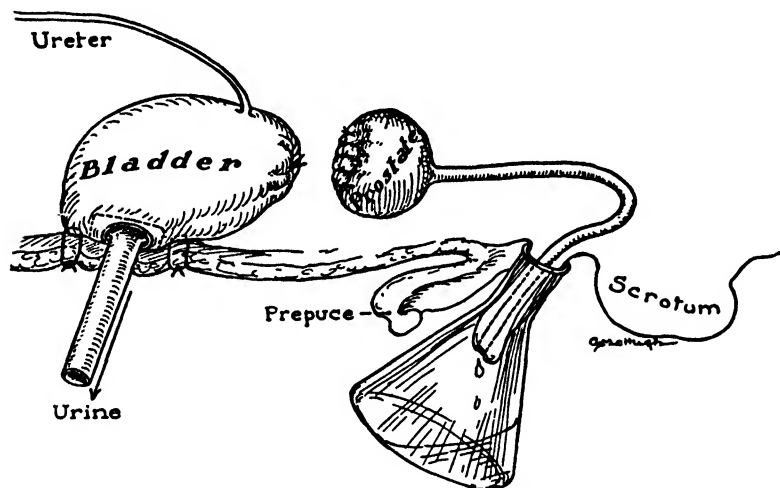

Fig. 1. Diagram of the prostatic isolation operation.

when tested at 2–day intervals with 6, 12, and 18 mg. of pilocarpine yielded 1.0, 8.1 and 26.1 cc. of fluid respectively.

The prostatic fluid is delivered from the urethra in a series of jets beginning from 1 to 12 minutes following the injection and the augmented secretion lasts for 50 to 90 minutes, more than 90 per cent of the secretion occurring in the first hour. Accordingly in most of these experiments fluid was collected for 60 minutes following an injection of pilocarpine-HCl, 6 mg. dissolved in 1 cc. of saline.

The prostate when in an active secretory state often eliminates

more than 2 to 4 times its weight in 1 hour or as much as 60 cc., indicating active secretory work. The greatly hypertrophied cystic hyperplasia of the prostate of senile dogs does not produce augmented amounts of secretion. The secretory volumes (71) are usually much less than are produced by a considerably smaller gland of a young adult.

The secretion frequently remains in a steady state for 3 months or more (Fig. 2), indicating that the intricate mechanisms for
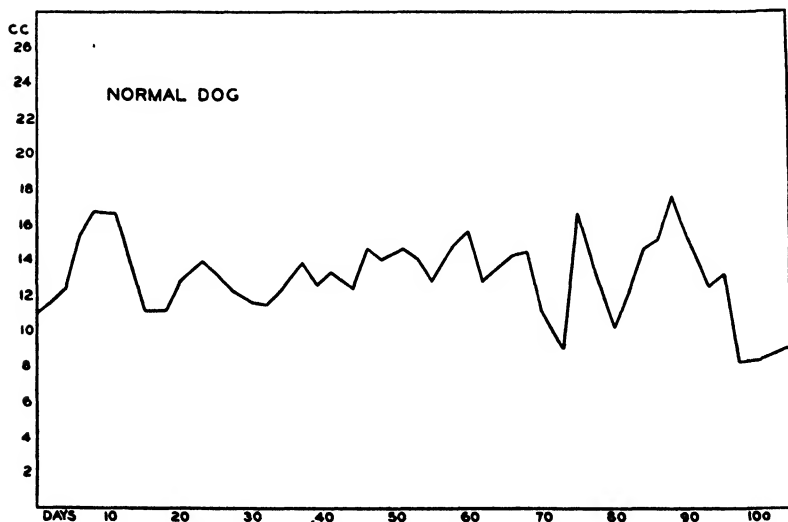
FIG. 2. Prostatic secretion of a normal dog. Ordinates, cubic centimeters per hour following injection of pilocarpine. Dog 331.

the production and destruction of androgen in the body as well as its utilization are in physiologic balance and in a steady state. Cyclic activity was not observed. At irregular intervals there were observed periods of depression or intense prostatic activity lasting about 7 to 10 days apparently reflecting fluctuation in the internal hormonal activity.

Infectious disease such as pyelonephritis or distemper often causes an abrupt decrease of prostatic activity where the secretion is abolished for many weeks before it recommences. Of in-

terest are disease states which cause secretory depression to a low level, for example an output of 3 cc. per hour (Fig. 3), without abolishing the prostatic function completely; this effect of systemic illness is due to inhibition of hormonal production by the hypophysis since injection of gonadotrophin will here augment the secretion (Fig. 4), signifying that the testis and prostate are relatively inactive but responsive. Similarly when the

FIG. 3. Decrease of prostatic secretion following distemper, the acute phase of illness lasting from Day 10 to Day 18. The output while greatly decreased was not eliminated. Abscissae, days. Dog 492.

secretion has been abolished by intercurrent infection gonadotrophin will sometimes restore prostatic activity but in the majority of instances the disturbance is more deeply seated and this stimulating agent is ineffective.

Effect of Androgen on Secretion. This is best studied in immature dogs or in castrate adults. With testosterone propionate[2]

[2] We are indebted to Dr. Erwin Schwenk of Schering Corporation for supplying generous amounts of this compound.

FIG. 4.   Decrease of prostatic secretion induced by distemper and overcome by injection of equine gonadotrophin, 20 units daily.   Dog 67.

dissolved in vegetable oil, prostatic secretion is demonstrable in puppies after 4 daily injections each of 5 milligrams at which time amounts of about 0.5 cc. of prostatic fluid, considered as unequivocal evidence of secretion, are obtained.   Subcutaneous or intramuscular injections are equally efficacious but intravenous injections have no effect, the material being inactivated in some way by the lung.

FIG. 5.   Prostatic secretion induced by varying amounts of testosterone propionate, daily dosage 5 to 25 mg. in castrate littermate dogs aged 4 months.

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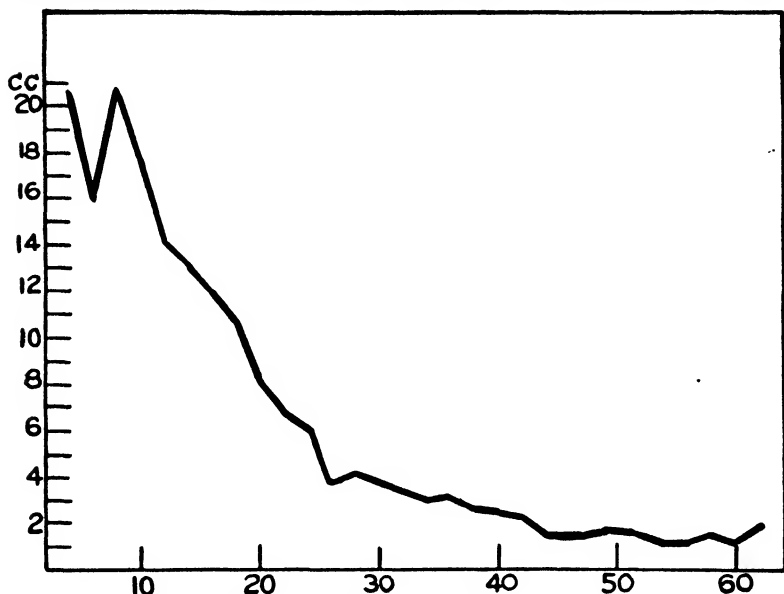tate are relatively inactive but responsive.   Similarly when the

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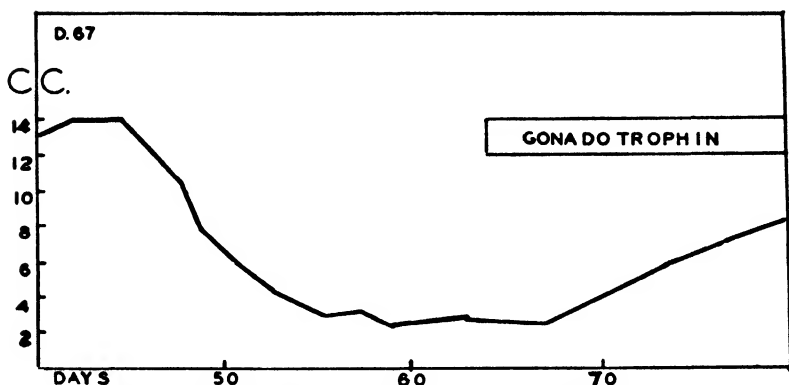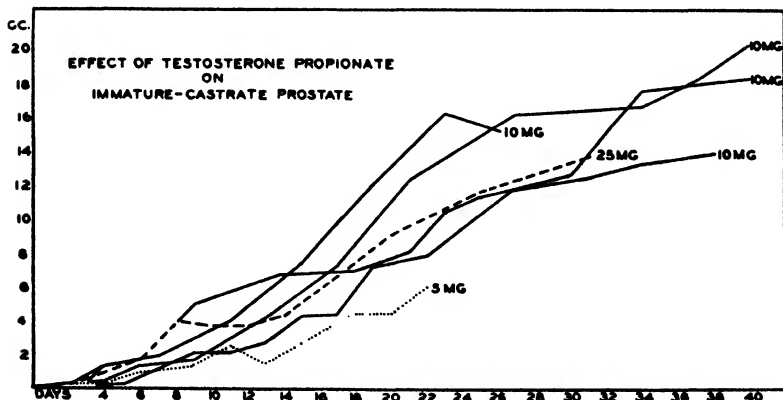
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FIG. 5.   Prostatic secretion induced by varying amounts of testosterone propionate, daily dosage 5 to 25 mg. in castrate littermate dogs aged 4 months.

In the initiation of secretion in dogs, essentially the same quantitative response occurs from 25 milligrams daily as from 5 milligrams (Fig. 5); apparently growth is maximal with the smaller dosage and increases of this selective growth-promoting agent do not considerably increase its rate. It was found by Deanesly and Parkes (7) that larger doses of testosterone did not make a proportionate difference in the size of the accessory glands of the rat. The secretory volume usually increases at a steady rate for very long periods of time without reaching a

TABLE 1

Utilization of Testosterone Pellets
Cylindrical Pellets Implanted Intramuscularly.
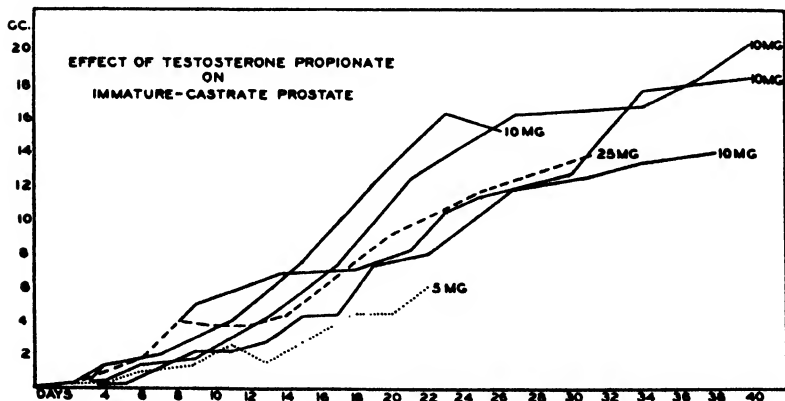
Dog	Weight of pellet	Duration	Daily utilization
	mg.	*days*	*mg.*
468	76	62	1.2
136	75.8	75	1.0
503	145	93	1.56
827	147	114	1.29
870	194	81	2.40
490	194	126	1.54
772	194	62	3.20
183*	75	101	0.74

* Pellet implanted in prostate.

plateau; in the castrate Dog 510, testosterone propionate 10 mg. daily induced a steady increment of secretion from 0 to 30 cc. in 6 months, the secretory curve continuing to rise during this time.

One might gain the impression from these remarks that whenever androgen and prostatic epithelium are present in a living creature, standard amounts of prostatic secretion result. I certainly wish to emphasize that more factors are involved. For example, occasionally the injection of androgen is ineffective in increasing the prostatic secretion of normal dogs for reasons which are unknown to me.

Effect of Implantation of Androgen Tablets. Deanesly and Parkes (7) discovered that increased physiologic effectiveness resulted when certain hormones were given to animals in the form of compressed pellets; in the rat absorption of testosterone from small pellets was from 0.07 to 0.15 mg. per day, and the accessories were maintained in normal condition from the daily absorption of 50 micrograms. In man, Shimkin and Zon (9) stated that diethyl stilbestrol yielded a constant average absorption of 0.35 to 0.45 mg. daily.

The isolation operation with subcutaneous implantation of cylindrical pellets of testosterone, 75 to 194 mg. in weight, re-
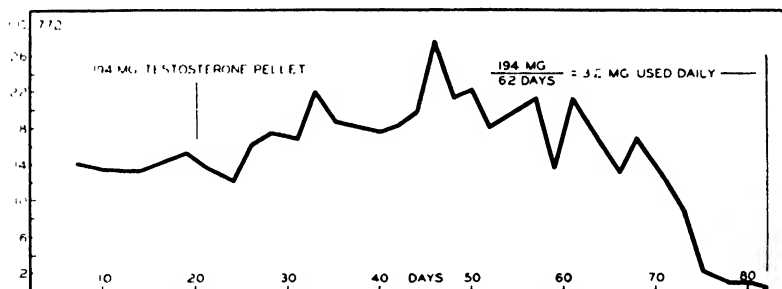
FIG. 6. Prostatic secretion following castration and implantation in muscle of testosterone, 194 mg. as a pellet. Dog 772.

vealed that on average 1.0 to 3.2 mg. were absorbed each day (Table 1). A pellet weighing 75 mg. implanted directly in the prostate gland of a castrate dog maintained secretion for 101 days (average absorption 0.74 mg.). Average results of utilization of steroid pellets however present only a first approximation, since absorption does not take place at a constant rate. Serial collection of prostatic fluid permitted the dynamics of absorption to be studied. Hormonal uptake from tablets did not occur at a level rate but always a prolonged rise in prostatic secretion was observed about the mid-period of androgenic effectiveness (Fig. 6). The rise is presumably due to an increased surface area because of irregular erosion of the cylinder.
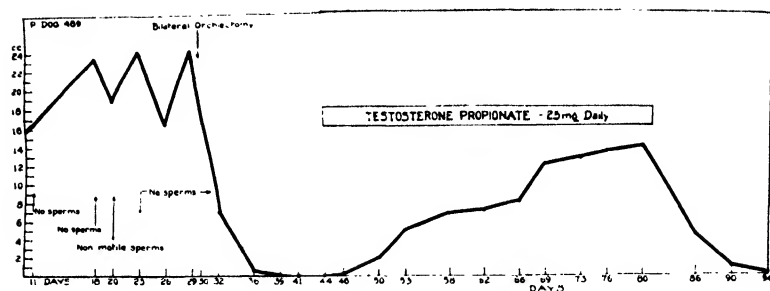
FIG. 7.   Elimination of prostatic secretion in a normal dog by orchiectomy and restoration by injection of testosterone propionate, 25 mg. daily. Dog 489.

Effect of Androgen Withdrawal.   Excision of the testes causes the prostatic secretion to decrease to one or two small drops of output (Fig. 7) within 7 to 23 days.   The average decrease in secretion is slightly lower in castrate dogs maintained on testosterone propionate dissolved in oil following the cessation of
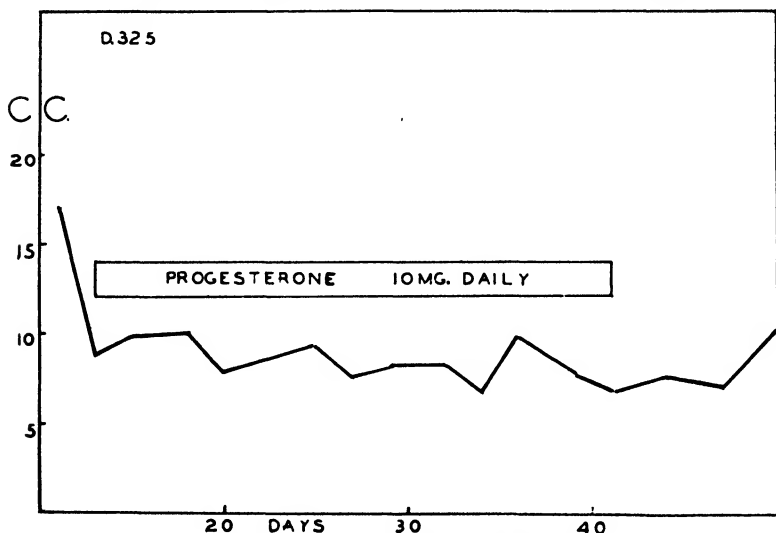
FIG. 8.   Lack of effect on prostatic secretion of injection of progesterone, 10 mg. daily for 37 days.   Dog 325.

treatment, the secretion being abolished in 16 to 23 days; no doubt some absorption from the injected oil prolongs the prostatic activity.

Effect of Progesterone and Desoxycorticosterone. Injections of progesterone, 10 mg. daily for 21 to 39 days, had no effect on the prostatic secretion (Fig. 8); in Dog 325 injected for 28 days the volume ranged from 8 to 9 cc. during this interval, and in Dog 432 the output was from 8 to 10.4 cc. in 39 days. Similarly, desoxycorticosterone acetate 5 mg. daily for 33 days (Fig. 9)
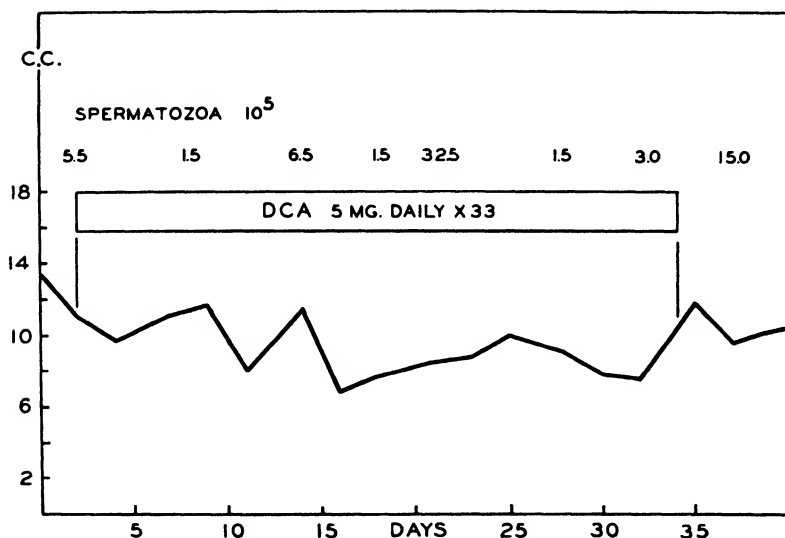
FIG. 9. Lack of effect on prostatic secretion of injections of desoxycorticosterone acetate, 5 mg. for 33 days. Dog 243.

caused no diminution in output. The chief interest in the observations is that these agents caused no androgenic antagonism or pituitary inhibition.

Effect of Estrogen on Secretion. In experiments done with Dwight R. Smith it was found that diethyl stilbestrol, 0.1 mg. dissolved in oil and injected daily, abolishes the prostatic secretion in normal dogs and causes squamous cells to appear in the prostatic secretion after 5–7 days (Fig. 10). These cells are

absent unless there is a dominating amount of estrogen present. The primary effect of estrogen on the male genital complex is depression of secretion of gonadotrophin by the pituitary as is shown in the following experiment.   Diethyl stilbestrol, 0.1 mg., was injected daily in a dog and when the secretion had ceased equine gonadotrophin[3] was injected, in addition, restoring the secretion (Fig. 10) and greatly decreasing the number of squamous cells in the prostatic fluid.   Substitution of this protein
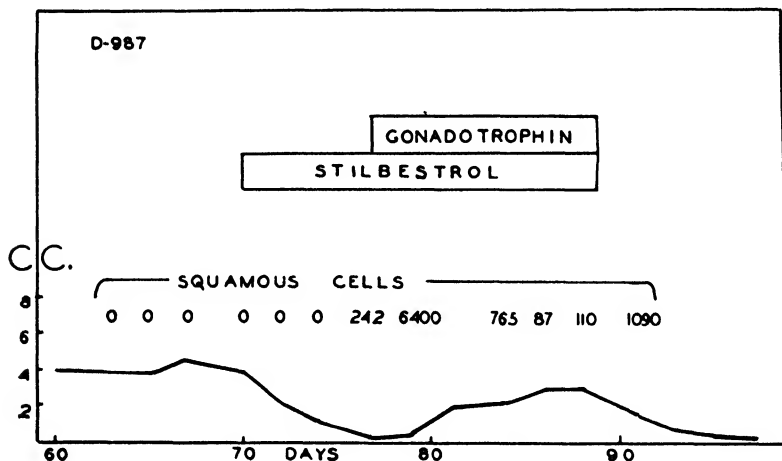
FIG. 10.   Elimination of prostatic secretion by daily injection of diethyl-stilbestrol, 0.1 mg. and its later restoration by treatment with equine gona-dotrophin, 20 units daily.   Squamous cells appeared in the prostatic secretion on the sixth day of estrogen therapy.   Squamous cells per cubic millimeter. Dog 987.

stimulant of the Leydig cells enabled them to produce enough androgen to stimulate the prostate equivalent to daily amounts of testosterone propionate at least 2.5 mg. as supplied by subcutaneous injection.

Estrogen also has the interesting capacity of largely neutralizing the effects of injected androgen (Fig. 11).   With continued

[3] Dr. E. Gifford Upjohn of the Upjohn Co. kindly furnished this material, sold under the trade name Gonadogen; 1 tablet contained 20 Cartland-Nelson units or approximately 400 International Units.

injections (71) of testosterone propionate, 10 mg. each day, diethyl stilbestrol 0.1 and 0.2 mg. daily have no effect on the rising secretory curve, but 0.4 mg. causes a plateau and 0.6 mg. causes a descending curve; thus the ratio of estrogen to abolish the stimulating effect of androgen is 1 : 25. The neutralizing effect of estrogen on androgenic activity resembles the metabolite inhibitors such as p-amino benzoic acid with respect to the sulfon-amide drug types.

The antagonism however is not complete. When androgen is injected with excess estrogen, the prostatic secretion is depressed
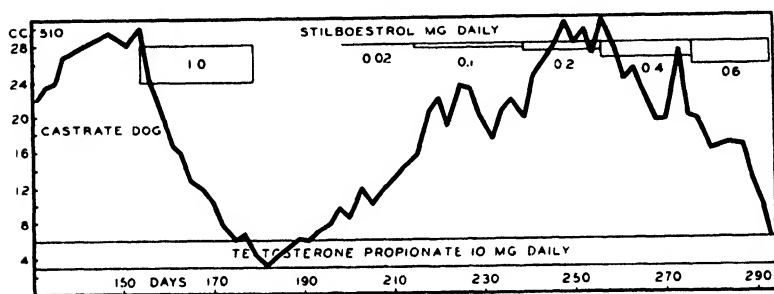
Fig. 11. Metabolite inhibition of testosterone propionate by diethylstil-bestrol. In this castrate immature dog, testosterone propionate, 10 mg. daily, was injected throughout the experiment and in addition stilbestrol injections were begun. Daily amounts of stilbestrol, 1.0 mg. and 0.6 mg. caused a great decrease of secretion while dosages of 0.02, 0.1, and 0.2 mg. did not affect the output. Dog 510.

to very low levels but is not eliminated; it decreases to about 0.5–2 cc. amounts but the acid phosphatase level is often more than 100 units comparable to the concentration of normal stimulated secretion. After long continuation of this regime (2–3 months) increasing the androgen does not promptly overcome the estrogen-induced secretory depression, as occurs with short-term experiments of 2–3 weeks. The failure to respond is no doubt due in part to organic changes in the gland.

Estrogen and the Functional Duality of the Prostate. The cytologic effects of estrogen on the prostate of dogs are illumi-

nating because of the varied response to it of two well-demarcated areas.   This functional difference permits recognition of the structural duality of this gland which has been believed previously to be homogeneous in the dog since in the gross the canine prostate is a single mass of tissue.   In one fraction of the gland, the dorsal segment, the epithelium undergoes squamous transformation (Fig. 12) after the threshold of response to estrogen has been exceeded, while the remainder of the prostate, the ventral
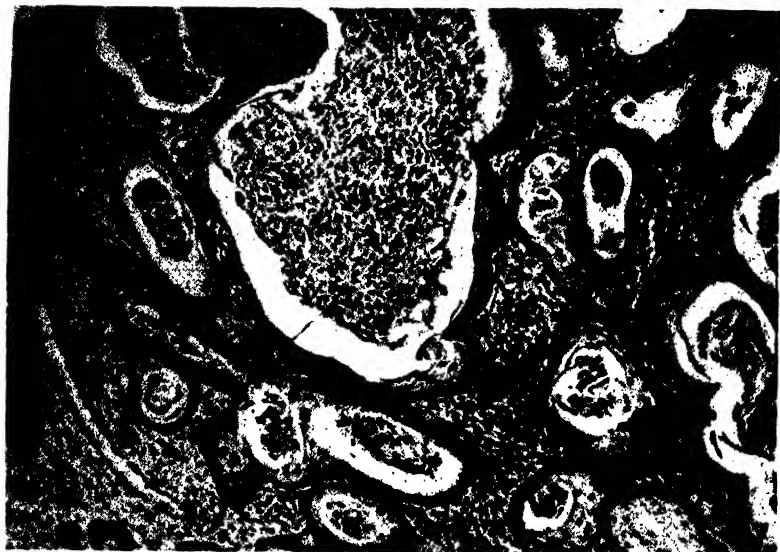
Fig. 12.   Squamous metaplasia of dorsal segment structures in a dog injected with stilbestrol, 1 mg. daily for 90 days.   Dog 750.   ×160.

segment, does not suffer this formation of squamous cells.   Both regions respond to androgen by developing tall columnar epithelium so that the difference between the segments in a normal adult is not apparent to common cytologic methods.   The epithelium of the whole prostatic urethra undergoes squamous metaplasia under estrogenic treatment, but that part covering the utriculus is more sensitive than the rest.   Excluding the urethral epithelium, the unit which forms squamous cells is: the veru

montanum, the prostatic ducts and the acini of the dorsal region of the prostate. The ventral two-thirds of the prostate never becomes squamous. If estrogen is given alone, the ventral region becomes atrophic and the acini are collapsed (Fig. 13) as a result of the complex wherein the animal is deprived of the androgenic

FIG. 13. Junction of ventral and dorsal segments of the prostate of a dog injected with stilbestrol 0.4 mg. daily for 29 days. Epithelium of the ventral segment (above) is atrophic while that of the dorsal segment is squamous. Dog 103. × 250.

stimulus. If both androgen and estrogen are administered in appropriate amounts, despite the great reduction of prostatic secretion, the ventral segment retains its columnar epithelium (Fig. 14) while the dorsal region is squamous. However, if a "threshold dosage" of estrogen is not present, androgen will cause both segments of the gland to respond by forming columnar

epithelium as in the normal dog.   Long continued administration of androgen and estrogen induces organic changes—vesiculation in the tall columnar epithelium of the ventral segment.

Studies of comparative anatomy substantiate this view of the duality of the prostate.   Walker (51) observed in the rat that only secretion from a specific region of the prostate, the anterior lobes (coagulating gland) would induce clotting of seminal vesicle
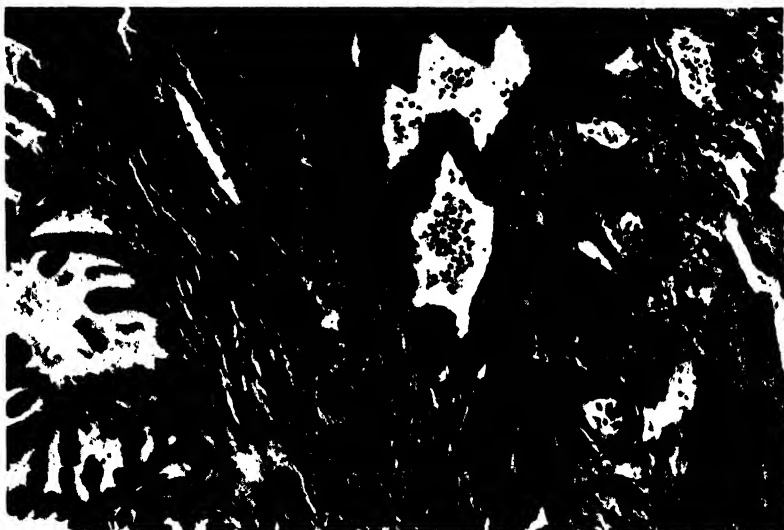
FIG. 14.   Junction of ventral and dorsal segments of the prostate of a dog injected with stilbestrol, 0.5 mg. daily and testosterone propionate 10 mg. each day.   Epithelium of the ventral segment (left) is columnar while that of the dorsal segment is squamous.   Dog 510.   × 125.

secretion, the other lobes being inactive.   This areal localization of clotting mechanism in the prostate occurs also in the guinea pig (51) and rhesus monkey (53).   The secretion of the anterior prostate of one species is able to coagulate the vesicle fluid of another (52, 53) but cross-coagulation is not so induced with human semen—using the vesicle fluid of rat or guinea pig as substrate.

In fine, the depressant effects of estrogen on the prostatic secre-

tion, when given in conjunction with androgen are due in part to transformation of the epithelium of the dorsal segment and partly are due to functional depression of the tall epithelium of the ventral prostatic segment.

CHEMICAL COMPOSITION OF PROSTATIC FLUID

The prostatic fluids of man and dog are slightly acid (about pH 6.4) and have a high water content, about 93 and 98 grams per cent respectively. They are remarkable for the small content of glucose (5, 37)—less than 30 mg. per cent. Despite histological similarity of the prostatic epithelium, the composition of the secretion differs considerably in these species. The prostate rarely does anything by halves; among the characteristics of the prostatic fluid is the great number of chemical effects among the enzymes and electrolytes which at first glance seem both surprising and interesting.

There are chemical differences in both dog and man between the spontaneous secretion without applied stimuli, *resting fluid* and that resulting from parasympathetic stimulation, *stimulated secretion.* Nearly all of the data on human prostatic fluid has been obtained on samples obtained by digital prostatic expression and is therefore *resting* fluid.

Electrolytes. (a) *Pilocarpine stimulated prostatic fluid of dog.* The concentration of osmotically active substances was 335 milliequivalents (m-Eq.) per kilo of water (5) of which the total base concentration consisted of sodium 162 m-Eq. and potassium 5.2 m-Eq. (Fig. 15), and calcium was present in very small amount, about 0.6 m-Eq. Among the anions the chloride concentration was 156 m-Eq. per kilo of water, total CO_2 between 1.6 and 1.8 m-Eq. and inorganic phosphate was present in traces only. Protein concentration was less than 1 gram per cent and citrate concentration 0 to 3 m-Eq. per liter.

(b) *Human prostatic fluid.* Only occasionally can amounts greater than 1 cubic centimeter be obtained from a single individual so that the chemical data necessarily represent a compilation from many subjects with the attendant dangers inherent in

statistical treatment. Median values for base per kilo of water (6) are as follows: sodium 156, potassium 30, and calcium 30 milli-equivalents (Fig. 15). The anions are chiefly citric acid 156 m-Eq. and chloride 38 m-Eq.; only small amounts of phosphate, 1 m-Eq., and bicarbonate, 8 m-Eq., are present. The pro-
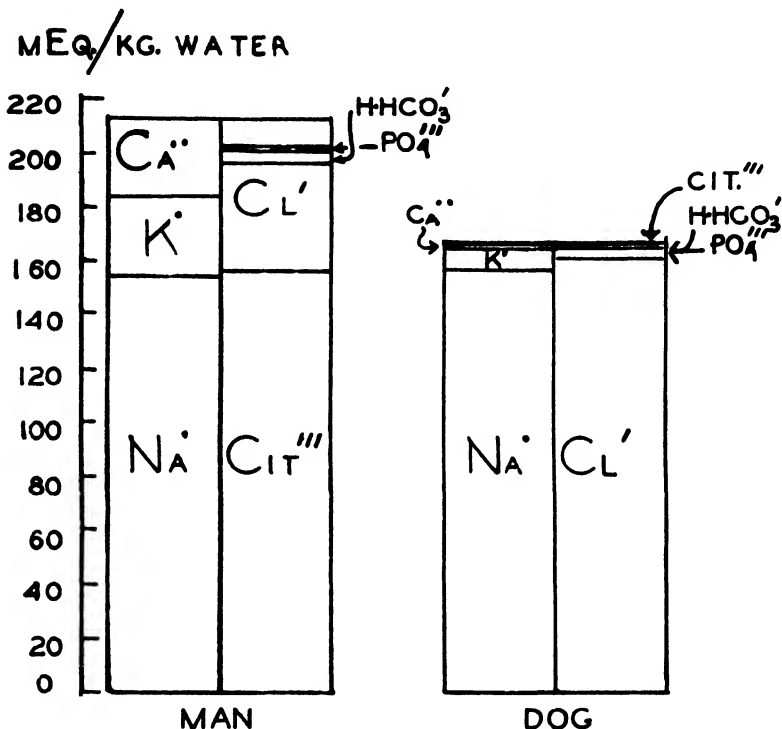
FIG. 15. Diagram of electrolytes in prostatic fluid.

tein content is about 2.5 grams per cent. The prostatic fluid does not coagulate on boiling, the proteins largely dialyze through cellulose membranes and so consist largely of proteoses.

Resting and Stimulated Prostatic Fluid and the Reciprocal Phosphatase Relationship. The chloride concentration (14) of resting prostatic fluid was 104 ± 22 m-Eq. per liter being one third lower than that of the fluid obtained from parasympathetic stimulation.

In the resting fluid of dogs (Table 2), the median value for acid phosphatase was 28 units per 100 cubic centimeters (14) while in stimulated fluid it was 104 units; the median value for alkaline phosphatase in resting fluid was 27 units while in stimulated prostatic fluid it was 2.25 units.   This reciprocal relationship of the

TABLE 2

Phosphatase in Prostate

King and Armstrong units per 1 gram of fresh tissue or 1 cc. of fluid.

	Acid phosphatase	Alkaline phosphatase
(a) Dog		
Prostatic fluid, resting (14)	0.28	0.27
Prostatic fluid, stimulated (14)	0.18–1.55	0.022
Prostate gland, puppies (69)	0.5 –1.02	0.27
Prostate gland, adult (30)	35.6	. . . .
Prostate gland, adult (69)	9.5 –26.5	0.4–26
(b) Man		
Prostatic fluid, stimulated (31) ...	1890–3950	. . . . ..
Prostatic fluid, resting (69)	117–1192	....
Prostate gland, new born (30)	1.5	....
Prostate gland, adolescent (30)	73	....
Prostate gland, hypertrophy (69)	668–4700	0.27–3.4
Prostate gland, adult (30)	522–2284	.. ..
Prostate, cancer, untreated (68)	19	... .
Prostate, cancer, untreated (69)	43– 188	....
Prostate, cancer, untreated (67)	8– 280	....
Prostate, cancer, treated with estrogen (67)	1– 20	... .

phosphatases was constantly observed and is explained by the different cellular distribution of these enzymes as will be discussed later.   Active secretion apparently washes out the small amounts of alkaline phosphatase from the epithelium rapidly while large amounts of acid phosphatase are held in reserve and are discharged on stimulation.

Most of the chemical analyses of human prostatic fluid have been done on the liquid obtained by digital expression of the prostate.   There exists however a method for collecting stimulated secretion in man ; it appears in nearly pure form in the first glass when the semen is collected serially (13) in 3 or 4 fractions. As in the dog, a considerable difference also exists between resting and stimulated fluid of man.   Acid phosphatase of prostatic fluid obtained during ejaculation in man ranged from 1890 to 3950
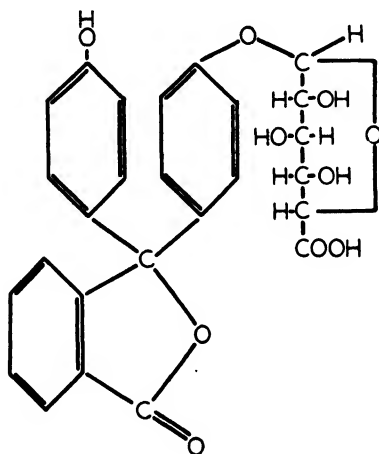
PHENOLPHTHALEIN
MONO·β·GLUCURONIC ACID

FIG. 16.

units (31) per cc.   The fluid obtained from 9 men in our clinic by prostatic expression ranged from 117 to 1192 units per cc. Obviously active prostatic secretion occurs during sexual stimulation and the ejaculate cannot be considered merely as a preformed secretion.

β-Glucuronidase.   The presence of this enzyme in prostate was demonstrated by the chromogenic substrate technique (54) using phenolphthalein glucuronic acid (Fig. 16) and measuring the amount of phenolphthalein liberated as an index of enzymic ac-

tivity.   Sodium phenolphthalein phosphate being a convenient water-soluble source of phenolphthalein was injected intramuscularly; it was hydrolyzed in the rabbit by phosphatase activity and was largely excreted in the urine as a phenolphthalein glucuronide from whence it was extracted by the method of DiSomma (55).

Spleen contains a greater concentration of glucuronidase than any other tissue, 25.3 Talalay units (54) per mg., but rat prostate is moderately high, 4.63 units per mg.

D. F. McDonald (70) found that glucuronidase content of human prostate gland was from 3 to 20 times greater than that found in the prostate of dogs and that human prostatic secretion was 10 to 40 times higher than canine prostatic fluid.

The significance of this enzyme is that it may represent a concentrating mechanism for hormones in target organs since the sex hormones are believed to circulate in the blood stream largely as water-soluble glucuronides.

Proteolytic Enzymes.   The semen of all animals is delivered from the urethra in a liquid state; in the dog it remains liquid while in man it coagulates to undergo subsequent liquefaction, most of the process being complete in about 15 minutes.   The mechanism of liquefaction was first discovered (20) by mixing prostatic fluid and blood which underwent clotting but soon liquefied.

Tillett and Garner (56, 57) observed that β-hemolytic streptococci contain a powerful proteolytic enzyme able to digest fibrin, which they named fibrinolysin.   Resistance to fibrinolysin occurs in certain people, especially those convalescent from streptococcal infections.   Recent evidence has indicated that streptococcal fibrinolysis is due to activation by the bacterial fibrinolysin (58, 59, 60) of an enzyme-precursor present in normal plasma with liberation of a protease which digests fibrin.

An enzyme nearly similar to streptococcal fibrinolysin was discovered in the prostatic fluid (20) and thus an agent previously only identified in association with bacteria was found to be a constant physiological constituent in man.   In prostatic fluid of

dog, the capacity to digest fibrin is much weaker than in the human secretion but a different activity, fibrinogenase, is present which digests fibrinogen powerfully. The proteolytic effects of prostatic fluid on blood and its clotting constituents will be considered further.

Human Prostatic Fluid. When equal quantities of freshly drawn blood and prostatic fluid are mixed, no clotting results because of the high citrate content of the secretion. When calcium ions are added in appropriate amounts, clotting occurs and more rapidly than in the control blood-saline tubes indicating a thromboplastic effect. Human semen contains fibrinogen and thromboplastin but not prothrombin or thrombin, as deduced (20) from mixing various constituents of the blood-coagulation process.

At 37°, 2 cc. of prostatic fluid will liquefy 100 cubic centimeters of human blood, when clotted, in 18 hours and occasionally it was powerful enough to liquefy a liter of clotted blood. Prostatic fluid lyses rabbit blood poorly and beef blood not at all.

Fibrinolysis occurs in the absence of cells and bacteria and is not reduced in amount in the cell-free fluid in chronic prostatitis (65). It is active (Fig. 17) against clots prepared by mixing a highly purified fibrinogen and thrombin. The blood of certain men is resistant to prostatic fibrinolysin and there is an immunologic difference between it and the streptococcal enzyme; the blood of humans resistant to the prostatic fibrinolysin has always been easily lyzed by the streptococcal enzyme and *vice versa* indicating an immunologic difference.

Prostatic Fluid of Dog. Differing from human prostatic fluid, this secretion in effective amounts has a thrombic activity; it constantly clots oxalated rabbit and beef plasmas (62) even in the presence of large excess of oxalate and heparin, although with heparin a flocculated precipitate develops instead of a firm clot; it cannot clot fibrinogen. The most striking characteristic is its ability to destroy fibrinogen (20) rapidly while it is relatively inactive against clotted blood (Fig. 17).

Many proteolytic properties of dog prostatic fluid resemble those of pancreatic trypsin. Furthermore, prostatic secretion of

dog constantly liberates chromomogen from denatured hemo-
globin at pH 7.5 (method of Anson (61)); prostatic fluid 1 cc.,
liberated 0.029–0.15 micromoles of trypsin in 15 minutes.   The
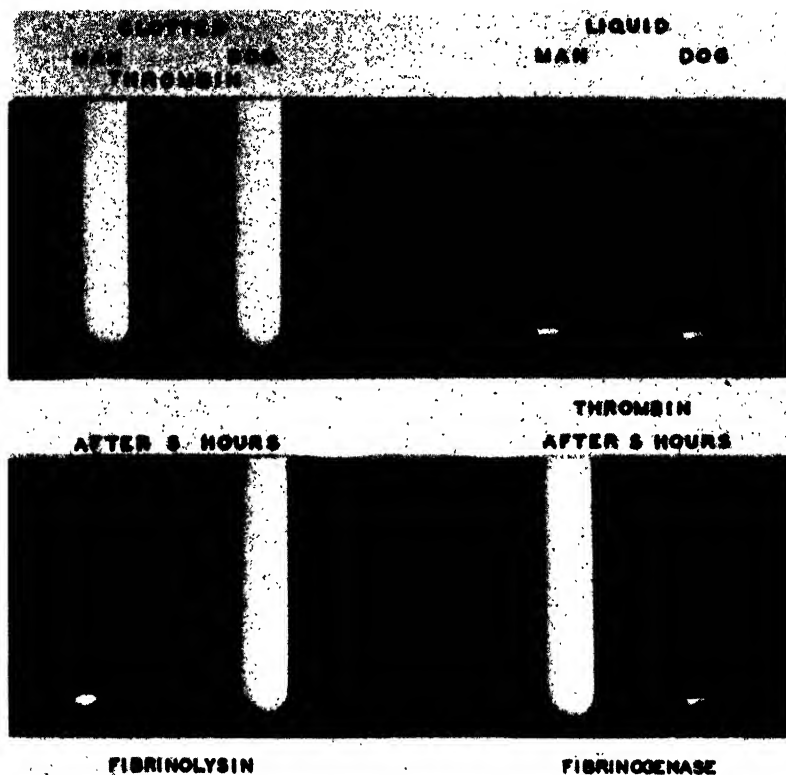
FIG. 17.   The difference between fibrinolysin and fibrinogenase.   Fibrin-
ogen and prostatic fluid were mixed; to demonstrate fibrinolysin thrombin
is added immediately and after 5 hours that tube containing human prostatic
secretion is liquid.   To demonstrate fibrinogenase (20) activity fibrinogen
and prostatic fluids were mixed and at 5 hours thrombin was added.   Dog
prostatic fluid inactivated fibrinogen while human prostatic fluid had no
fibrinogenase activity so that only this sample clotted.

prostatic secretion of dog differs from trypsin in that it destroys
fibrinogen much more rapidly than fibrin.   The smallest amounts

of dog prostatic fluid which induced clotting of rabbit plasma destroyed fibrinogen in 5 hours while the smallest amounts of trypsin effecting coagulation did not digest this protein.   The crystalline

TABLE 3

*Action of Trypsin Inhibitors on Trypsin and Dog Prostatic Fluid Fibrinogenase**

Tubes were incubated one hour at 37° C. before addition of fibrinogen. Final volume of each tube was made up to 4.0 ml. with 0.9% saline.

Fibrin- ogen, ml.	Trypsin, mg./ml.	D.P.F., ml.	Trypsin inhibitor, mg./ml.		Time of destruction of fibrinogen
			Soy bean	Pancre- atic	
0.625	0.02†	....	0	... ...	6 minutes
0.625	0.02	... .	3.5	... .	Longer than 24 hours
0.625	0.02	....	10.5	....	Longer than 24 hours
0.625	0.011‡	....	....	0	1 minute
0.625	0.011	....	......	0.0037	Longer than 24 hours
0.625	0.022	....	....	0.0037	1 minute
0.625	0.022	. ...	....	0.0073	Longer than 24 hours
0.625	....	1.25	0	....	5 minutes
0.625	....	1.25	0.5	....	10 minutes
0.625	....	1.25	1.0	....	45 minutes
0.625	....	1.25	5.0	....	180 minutes
0.625	....	1.25	....	0	5 minutes
0.625	....	1.25	....	1.25	25 minutes
0.625	....	1.25	....	2.50	45 minutes
0.625	....	1.25	....	12.50	120 minutes

* Experiment done with Doctors D. F. McDonald and I. A. Mirsky.
† 1: 250 Trypsin Difco Laboratories.
‡ Crystalline trypsin of Kunitz.

"trypsin inhibitor" of pancreatic origin (63) and the anti-trypsin derived from soy-beans (64) completely inhibited the action of trypsin on fibrinogen but did not inhibit canine or human prostatic fibrinolytic or fibrinogenase activities (Table 3)

even in large amounts.   The principal proteolytic activity of dog prostatic fluid, fibrinogenase, resembles but is not identical with trypsin.

SECRETORY MECHANISMS

An analysis of the distribution of the constituents of canine prostatic fluid obtained by stimulation gives a clue to secretory mechanisms.   Several experimental conditions were set up by modifying the time and the intensity of the stimulus.   In castrate dogs injected with androgen at constant rate, the secretion was collected for consecutive periods of 15 minutes after pilocarpine administration; (a) intravenously, (b) intramuscularly, (c) repeated injections.   Intramuscular administration of pilocarpine stimulates the gland more slowly than intravenous injection because the drug reaches the gland more slowly.   Therefore by these means one can distinguish between a "washing out" of the cells from secretory processes of a more active type.

(a) *Single intravenous injection.*   Maximum values were obtained in the first period (15 minutes) after injection of pilocarpine for the following items: (1) volume, (2) total protein concentration,[4] (3) the enzymes, acid phosphatase, glucuronidase, fibrinogenase, (4) citrate.   The three succeeding quarter-hour periods always showed progressively declining values for all of these categories as compared to the initial collection.   In an experiment on Dog 991 citrate excretion in the first period was 30.5 mg. per cent, in the second 15 minutes 0.9 mg. per cent, and then there was no more citrate excretion.

[4] The following methods were used: Protein was estimated by multiplying by 6.25 the total nitrogen corrected for non-protein N; acid phosphatase (32); glucuronidase (54); citrate (66).

Fibrinogenase activity was determined by an unpublished method of the author.   9.5 cc. of fibrinogen solution composed of 1 gram in 100 cc. of M/30 phosphate buffer at pH 6.6 are warmed to 37°; to this mixture 0.5 cc. of prostatic fluid is added at 0 time.   At 4-minute intervals 0.5 cc. of the mixture is withdrawn and added to a tube containing 2 drops of thrombin, 10 mg. dissolved in 10 cc. of water.   Time is the end point; the fibrinogenase activity is that time in which no clotting occurs signifying inactivation of fibrinogen.

On the contrary, chloride always rose initially from the low values of the resting secretion and reached its maximum (Fig. 18)
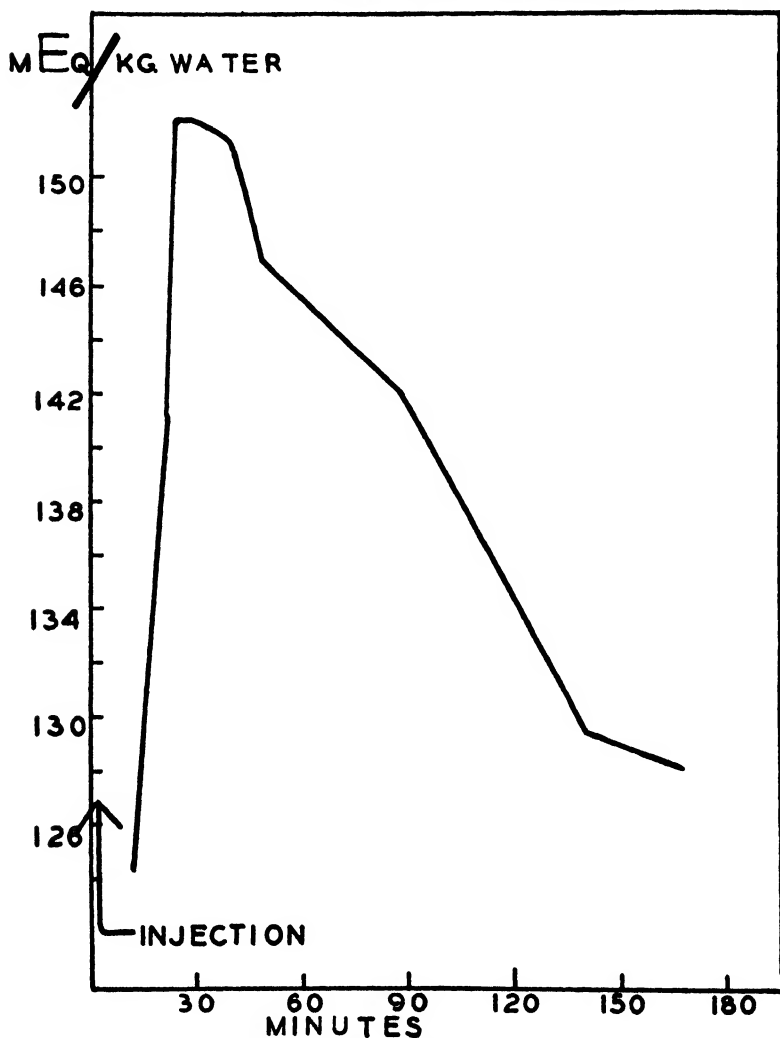
FIG. 18.   Chloride excretion in serial fractions following intravenous injection of pilocarpine hydrochloride 6 mg.   Dog 991.

after the first period.   The collection of prostatic fluid in 2-cc.
amounts as delivered at frequent intervals during the secretory
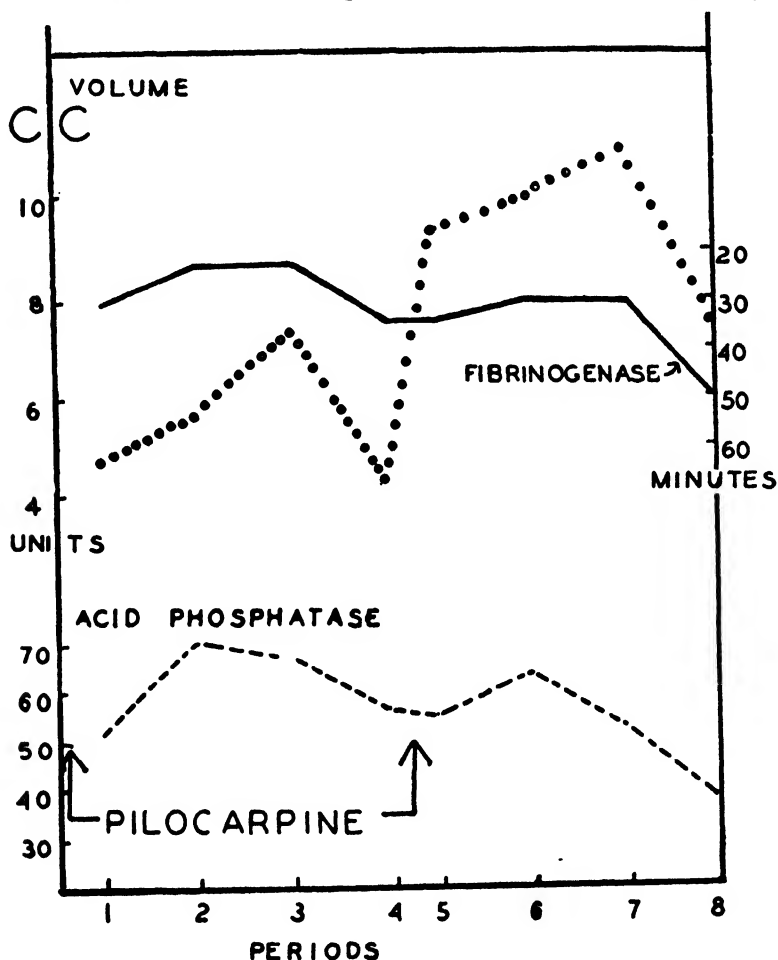
FIG. 19.   Repeated intramuscular injections of pilocarpine 6 mg. at 0 and
60 minutes with collection of prostatic fluid for periods of 15 minutes dura-
tion.   Intramuscular injection causes a delayed maximum excretion of fibri-
nogenase (————), acid phosphatase (·······) and volume (° ° ° ° °).
Dog 991.

process permitted serial chloride analyses which revealed that the maximum concentration was attained in 22 to 40 minutes (median 30 minutes), and high values persisted for about 90 minutes after injection.

(b) *Intramuscular injection.* This route provided a delayed secretory stimulus (Fig. 19) since the drug came to the gland more slowly. Maximum values were obtained in the first period
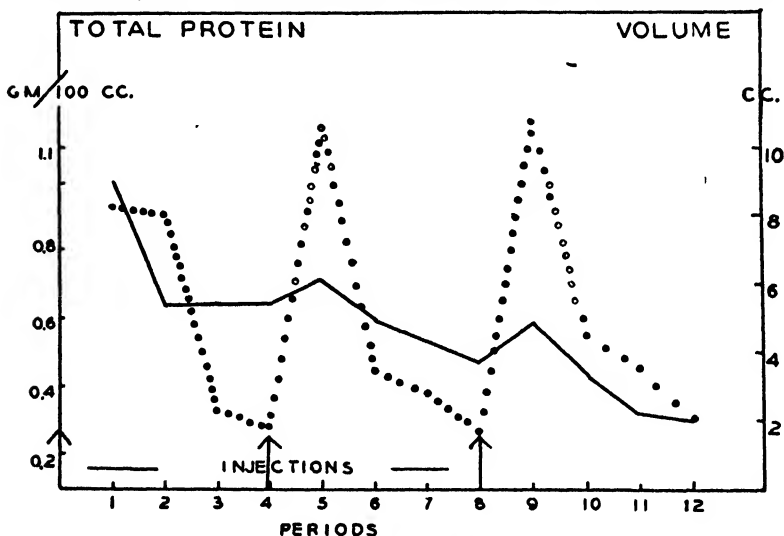
FIG. 20. Repeated intravenous injections of pilocarpine 6 mg. at 0, 60 and 120 minutes with collection of prostatic fluid for periods of 15 minutes duration. Total protein (————) gradually decreased but there were spikes of increment following each injection. The volume (° ° ° ° ° °) of prostatic secretion increased after each injection. Dog 991.

only for total protein concentration and citrate. Otherwise the values were higher in the second and third periods for the volume, chloride concentration and the enzymes tested.

(c) *Repeated intravenous injection.* Here the gland was stimulated sharply two or three times at hourly intervals, collecting the secretion in 15-minute periods as before; fluids were not administered during the testing. In general there was a decreased concentration of protein (Fig. 20) and also the enzymes

acid phosphatase and fibrinogenase during the course of the experiment (Fig. 21) except that each intravenous injection pro-
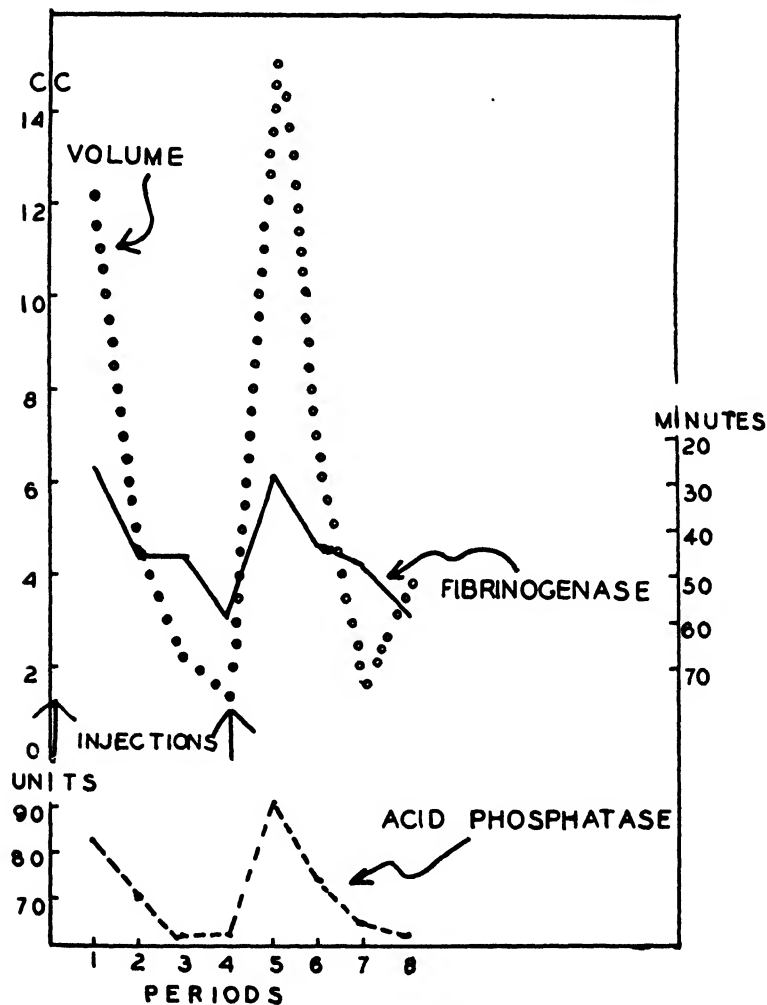

FIG. 21. Repeated intravenous injections of pilocarpine, 6 mg., at 0 and 60 minutes. Increases of volume (°°°°°°), fibrinogenase (————) activity and acid phosphatase (------) followed each injection promptly.

duced a slight but definite increment interrupting the otherwise steady decrease. The protein concentration in some of the experiments of three hour duration decreased from 1.7 grams to 0.25 grams in 100 cubic centimeters.

Several deductions may be drawn from these data, namely: (1) a "washing out" process occurs in the cells after an initial stimulus with respect to total protein and citrate concentration, since both stronger and weaker stimulation of the gland result in maximum secretion of these constituents early in the course of secretory events; (2) repeated sharp stimuli of cellular secretion in the form of repeated intravenous injections of pilocarpine cause slightly increased secretion of the enzymes acid phosphatase, glucuronidase and fibrinogenase interrupting their downward trend; these enzymes and chloride are definitely secreted and not merely washed out; (3) chloride concentration is dissociated from volume increments and the secretion of the various protein constituents. Intravenous injections of pilocarpine show that prostatic secretion is not the attempt of the prostatic cell to eliminate chloride because the greatest volumes are eliminated long before the maximal chloride concentration is reached in the fluid.

Bucher and Ivy (8) studied the effect of the "double histamine test" on the secretion of a pouch of the stomach in dogs with respect to volume and concentration of chloride and pepsin. Both the concentration and output of pepsin increased in response to each injection of histamine.

Clearly in the prostate stimulation causes both elimination of preformed products such as citrate and protein and also an increased secretion of other fractions—chloride and certain enzymes. It cannot be decided whether the latter proteins were synthesized during the experiment or were preformed and held in reserve, the gland parting with them only when presented with a strong stimulus.

Available Body Water and Prostatic Fatigue. In addition to stimulating the prostate, pilocarpine causes salivation, lacrimation, and at times diarrhea with loss of body water. This loss in

itself is insufficient to stop prostatic secretion although the available water in the internal environment does have its influence on secretion.

Two types of prostatic response to pilocarpine injections at short intervals have been observed; the prostate gland of some dogs became more "fatigued" than others—this fatigue is recognized as yet only by decreases of volume.

A "strong secretory" gland acts as follows: When pilocarpine 6 mg. is injected intravenously and repeated at the end of one hour, the secretion during the second hour is greater in volume than that of the first hour; the second stimulus may be repeated after a rest of 2 to 4 hours and still the gland is hyper-excitable but the increased irritability has always passed off at 6 hours. These "strong glands" usually are seen in dogs injected with androgen or those with a large spontaneous secretion.

Less often "weakly secretory" glands are encountered; when pilocarpine 6 mg. is injected and repeated at the end of one hour, the secretion during the second hour is less than that of the first collection. If the dosage of pilocarpine is doubled for the second injection or if a liter of sodium chloride 0.15 N or glucose 5% is given intravenously before the second testing, secretion of the second hour will exceed the first. This indicates that the potential for strong secretion is present. Therefore in "weak glands" a diminished response to a standard stimulus occurs which is not necessarily related to dehydration since a strong second stimulus is effective and results in an augmented volume.

Fatigue effects are observed in the prostate and seminal vesicle in man where ejaculation at very short intervals results in a great decrease in the volume of the ejaculate as well as in the amount of protein secretion (6): here dehydration does not enter the picture. In an observation, which is typical of usual events, in a man, A.M., the normal semen volumes of 4 cc. decreased when ejaculation was repeated in 1 hour to 1 cc. and at 2 hours to 2 cc.: recovery to the original 4 cc. volume occurred at 24 hours.

Effect of Starvation. Moore and Samuels (10) found in rats that a diet insufficient in calories resulted in prostatic atrophy

which however could be overcome by injection of testicular extracts. Mulinos and Pomerantz (11) also fed diets which were qualitatively adequate but were reduced to about one-half of the amounts required for growth of the prostate of the rat: those observers found that chorionic gonadotrophin or testosterone caused a prompt and considerable increase in weight of the prostate. This underfed condition they regarded as pseudo-hypophysectomy.

These experiments were repeated (12) under the more rigorous conditions of complete withholding of food for three weeks but with free access to water. About 4 days after the onset of starvation there was a decrease of prostatic output which continued until secretion was abolished 3 to 12 days later. Gonadotrophin injected on the 16th day of starvation restored the secretion. Testosterone propionate induced greatly increased secretory volumes which returned to a lower level when feeding was recommenced.

The hyper-excretion in starved dogs injected with androgen was not a systemic effect since the secretion of the saliva collected from a salivary fistula decreased steadily during inanition; it may well be related to decreased inactivating mechanisms since starvation causes a decreased inactivation of certain steroids, such as estrogen, apparently through disturbed liver function.

Testosterone propionate likewise caused growth of the prostate of starving, immature dogs and the prostate weighed 390 to 468 per cent more in the injected animals than in the litter-mate controls, although the dogs lost 22 to 33 per cent of the body weight. There was a 50-fold increase in acid phosphatase content. Thus androgen in the complete absence of alimentation caused growth with protein synthesis in this unessential structure during a period of potential danger.

Lipids. Sir Henry Thompson (15) first observed that "yellowish refractile bodies about 1/5000 to 1/2500 of an inch in diameter are always present in prostatic fluid and in certain prostatic cells." These bodies are lipids which possess double refraction in polarized light (38, 39). Lipids have been frequently demon-

strated in the prostatic cells and their presence in the prostatic fluid represents a true secretion. The total lipids of canine prostatic fluid (69) range from 30–40 mg. per cent.

Scott (16) found that the total lipid content of human prostatic fluid averaged 0.286 grams per hundred milli-liters of which about 63 per cent were phosphatides mostly cephalin, and 28 per cent cholesterol. Neutral fat, lecithin or 17-ketonic steroids were not present. Cholesterol participates in the formation of calculi.

Citrate and the Metabolism of the Prostate. From the standpoint of oxidative metabolism the prostatic gland belongs with tissues possessing a low oxygen uptake like the adrenals, resting submaxillary gland and thymus. Human prostatic adenoma (17) possesses a low rate of respiration (Q_{O_2} 1.95), a high anaerobic glycolysis ($Q_L^{N_2}$ 5.39) and a considerable aerobic glycolysis ($Q_L^{O_2}$ 2.21); the respiration of normal prostate of dog and rabbit is more than twice as large. The oxygen uptake of human prostatic adenoma was increased on addition of succinate, pyruvate and citrate.

Biopsy of the prostate of dogs before and after castration or estrogen showed that the anti-androgenic measures cause a diminished oxygen uptake in slices of prostate *in vitro*, a diminished pyruvic oxidation without disturbing the anaerobic phase of carbohydrate utilization (Table 4). These results indicate either that androgen takes part in the direct regulation of the oxidative phase of carbohydrate metabolism of prostatic tissue or more likely, that the activity of the respiration process is altered as a response to the changing energy demands induced by androgenic or estrogenic therapy in the gland.

The human prostate is remarkable for its high content of citric acid (18, 19, 20); (expressed in grams for 100 grams, the secretion 0.48 to 2.68; benign hypertrophy 0.218 to 1.533; cancer 0.012 to 0.137) while the canine prostate has low citrate values (secretion 0–0.03; gland 10 mg. for 100 grams). These relationships were further investigated (21) with Dr. E. S. Guzman Barron. Citrate and α-ketoglutaric acid are synthesized but not utilized by the human prostate *in vitro* so that citric acid ac-

cumulates; both formation and destruction of citrate were demonstrated in slices of the canine prostate.

The synthesis of citric acid by heart, kidney and liver *in vitro* has been well established, the required substances being oxaloacetic and pyruvic acids. Synthesis of citric acid by the prostate of dog was as vigorous as that by chopped rat heart while human prostatic adenoma under the same conditions yielded only one-seventh as much citric acid; on incubation in pyruvate and

TABLE 4

Effect of Orchiectomy and Estrogen on Metabolism of Dog's Prostate
The Q values give c.mm. per mg. dry weight per hour

Dog No.	O$_2$ uptake				Lactate formation				Pyruvate utilization	
	QO$_2$ glucose		QO$_2$ pyruvate		Q$_L^{N2}$		Q$_L^{O2}$		Q pyruvate	
	Before	After	Before	After	Before	After	Before	After	Before	After
(a) orchiectomy—31 days										
809	4.26	2.21	5.74	4.28	4.0	4.74	1.05	2.74	3.12	3.0
(b) stilbestrol, 24 mg. in 28 days										
827	4.11	3.14	5.0	3.45	6.2	7.0	0.69	1.84	7.6	2.08

fumarate dog's prostate produced about 0.46 mg. citric acid per gram in 30 minutes.

The prostate of both dog and man contain vigorous transaminating mechanisms which permit the formation of oxaloacetate. The two main transaminating reactions of Braunstein and Kritzmann (22),

(1) glutamate + oxaloacetate $\rightleftharpoons$ aspartate and + ketoglutarate
(2) glutamate + pyruvate $\rightleftharpoons$ alanine and + ketoglutarate

were studied in human prostatic tissue slices. The rate of transamination was vigorous; in these experiments equilibrium was reached in 30 to 60 minutes in all cases.

Another enzyme, aconitase, was extracted (23) from human prostate in rich amounts, the dog's prostate yielding only about half as much.   Aconitase is the enzyme responsible for conversion of cis-aconitic acid to citric acid.   The aconitase content is 60 per cent as high as pigeon breast muscle, about equal with kidney and 10 times as high as testis.

In summary, *in vitro* experiments show that human prostate is capable of synthesizing citric acid much faster than it can oxidize it while the dog prostate utilizes it much more readily and in the latter case the rate of breakdown more nearly equals the rate of synthesis.   Transaminating mechanisms in prostate are one source of oxaloacetic acid and condensation of this dicarboxylic acid with pyruvic acid would give cisaconitic acid which in the presence of aconitase would be hydrated to citric acid.   The existence of powerful transaminating mechanisms and aconitase in human prostate speak for this scheme.

Calculi—Difficultly Soluble Products of Secretion.   Concentrically laminated spheroidal bodies the corpora amylacea stated to be composed of protein and nucleic acid (24) occur very frequently in the lumina of the prostate of adult man and anthropoids but never in this gland in the dog or rat.   They frequently become calcified, radio-opaque and of large size and under these circumstances accumulate largely in the prostatic ducts.   Advantage may be taken of these facts to demonstrate the pathway of these structures; the larger ducts (25) course between the peri-urethral lobes and the posterior lobe to approach the midline in a crescentic manner.

The chemical composition of prostatic calculi was determined (25) in 10 patients and it varied over a narrow range.   The organic components amount to about 21 per cent and include protein, 8 per cent; cholesterol, 7.13 per cent; and citrate 2.3 per cent.   The greatest fraction of the stone is inorganic (Table 5) and consists of calcium and magnesium phosphates and carbonates.   Dried prostatic calculi and rib bone of the calf in x-ray diffraction patterns revealed essentially similar principal spacings and substantiated the analytical data that the chief constituent of

prostatic stones is tertiary calcium phosphate, $Ca_3(PO_4)_2$ arranged in apatite crystal structure. Carbonate is somewhat less than in bone but otherwise the inorganic composition is similar.

It is of interest that all of the constituents difficultly soluble in water precipitate or are adsorbed when calculi form.

Phosphatases in Prostate. Acid phosphatase was characterized independently by Davies (26), and Bamann and Riedel (27) in 1934 and was found to be present in very large amount in the prostate gland of man (28) and rhesus monkey (29). Gutman and Gutman (30) found that the prostate gland of children con-

TABLE 5

Chemical Analysis of Prostatic Calculi
Mg. per 100 mg. of dry powdered stone.

Calcium	Magnesium	Phosphorus as PO₄	Carbon dioxide	Citric acid	Protein N × 6.25	Cholesterol	Total	Ash
30.3	2.89	55.5	1.27	2.30	8.07	7.13	107.7	79

Average values of stones from 6 patients.

tains very little acid phosphatase, 1.5 units[5] per gram of fresh tissue at 4 years; it increases however during puberty (73 units) to very high values in the adult (522–2284 units per g. fresh tissue). In our laboratory the acid phosphatase content in the hypertrophied prostate glands of men ranged from 668 to 4700 (median 1745) units per g. of fresh tissue; in the prostate of puppies, (73) acid phosphatase varied from 0.35 to 1.73 units while in the adult dog it ranged from 18.6 to 70 units per gram. Gutman and Gutman (35) observed that testosterone propionate caused a several hundred-fold increase in acid phosphatase activity of the puberal monkey prostate gland increasing it to adult levels.

Alkaline phosphatase in the prostate of immature (12, 73) and adult dogs and of man lay between 0.27 and 3.4 units per g. of fresh tissue.

[5] All phosphatase units are those of King and Armstrong (32).

The cellular distribution of the phosphatases was elucidated by Gomori (33, 34) who devised methods for staining the locus of acid (1941) and alkaline phosphatase (1939) in tissue sections. The pattern is entirely different. Alkaline phosphatase occurs chiefly in the walls of capillaries although some may be found in the acinar epithelium while acid phosphatase is only present in the epithelial cells and in the secretion in their lumina.

Now a few words about the function of these enzymes in the prostatic fluid. Clearly if appreciable amounts of substrate were to enter the prostatic lumina, the phosphatases would cause them to turn rapidly to stone because of the high calcium content of prostatic fluid and its weakly acid pH; in this regard it is well to consider that small or large prostatic calculi are encountered frequently in the human prostate.

Lundquist (74) has obtained evidence that the seminal vesicle elaborates considerable amounts of phosphoryl choline which is readily hydrolyzed by the prostatic enzymes with liberation of choline and inorganic phosphorus; this is the only function of acid phosphatase established as yet.

Phosphatase Excretion in Urine. It has long been known that human urine contains phosphatases (54) of which the largest amount is acid phosphatase (46, 47, 48). The urine of normal adult males contains 3- to 5-fold greater amounts of acid phosphatase (49) than that of normal adult females or children. Much evidence shows that the enzyme is derived mostly from the prostatic secretion. Thus, the amounts of acid phosphatase in prostatic fluid is very large, and the urine contains large amounts after sexual stimulation. In the "two glass test" in the young adult male (49) there is consistently more phosphatase in the first ounce of urine voided than in the urine which follows. Urine obtained from the kidney (49) by catheter contains small amounts of phosphatase while voided urine contains large amounts. Also castration produces marked decreases in the urinary concentration of this enzyme.

The high acid phosphatase of the urine of human males then depends upon an admixture of prostatic fluid so that this enzyme

may serve as an index of prostatic secretion. It was of interest to find that the daily output of acid phosphatase in human urine was nearly constant for long periods of time indicating a uniform rate of insensible prostatic secretion.

Older methods for phosphatase assay in urine were somewhat tedious since the urine required dialysis to eliminate interfering phenols or phosphates. A simplified assay technique (50) has been developed making dialysis unnecessary; in this technique sodium phenolphthalein phosphate is used as a substrate, the amount of phenolphthalein liberated by enzymic scission serving as an indicator of phosphatase activity.

Prostatic Secretion in the Blood. Acid phosphatase does not enter the blood from the prostate in its normal situation; however, this occurs when prostatic tissue becomes ectopic as cancer and involves certain regions of the body such as bone marrow and lymph glands—selective locations where it flourishes. The Gutmans (36) made the important discovery in 1938 that acid phosphatase is often increased in the serum of patients with diffuse prostatic carcinoma. Acid phosphatase does not readily cross certain membrane barriers; it is low in amount in the cerebrospinal and spermatocele fluids in cases with high serum levels (41). Apparently there is a greater permeability in bone marrow and lymph node than in the prostate in its normal site which permits the entry of proteins the size of the phosphatase enzymes from those regions into the blood.

While fluctuating values occur the general tendency of acid phosphatase is to rise during the natural course of prostatic carcinomatosis in a metastatic state; sometimes however identical values are observed for several weeks in a given patient indicating a delicate balance of input and outgo of prostatic secretion from the cancer into the blood. The injection of acid phosphatase (human semen) intravenously in dogs indicates that the enzyme is rapidly withdrawn from the blood stream with return to normal value within 3 to 6 hours after administration (Fig. 22). In addition metastatic prostatic cancer in bone often causes osteoblastic proliferation (mechanism uncertain at present) and in

these cases the alkaline phosphatase of serum is considerably increased as was discovered by Kay (43).

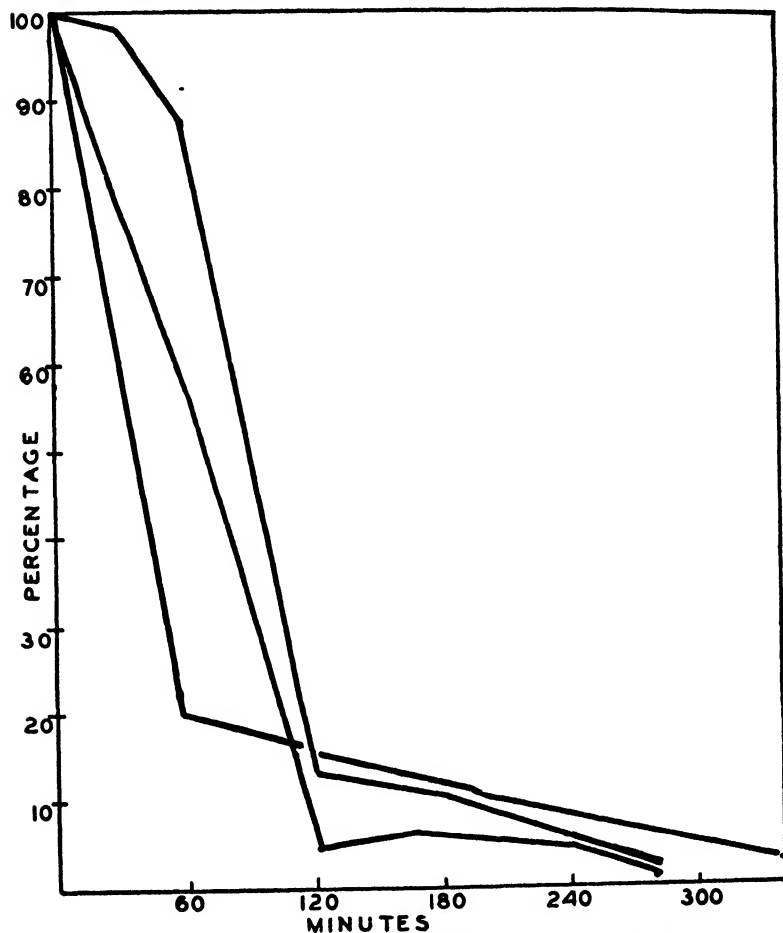Cancer of the prostate contains less acid phosphatase than the

FIG. 22. Withdrawal rates of acid phosphatase from the serum of dog. At 0 minutes 2 cc. of semen (c. 5000 units) were injected intravenously and the first sample of blood was withdrawn 4 minutes later; this value was designated as 100 per cent and subsequent values are given in relationship to it.

normal gland—approximately one-twentieth; Gutman, Sproul, and Gutman (68) found that acid phosphatase content of an osteoblastic metastasis in bone was 19 units per gram. In our laboratory 8 malignant prostates contained acid phosphatase 93 to 188 units per gram and alkaline phosphatase 0.5 to 1.28 units (Table 2). The citric acid content (23) likewise is considerably less in malignant disease than in benign hypertrophy.
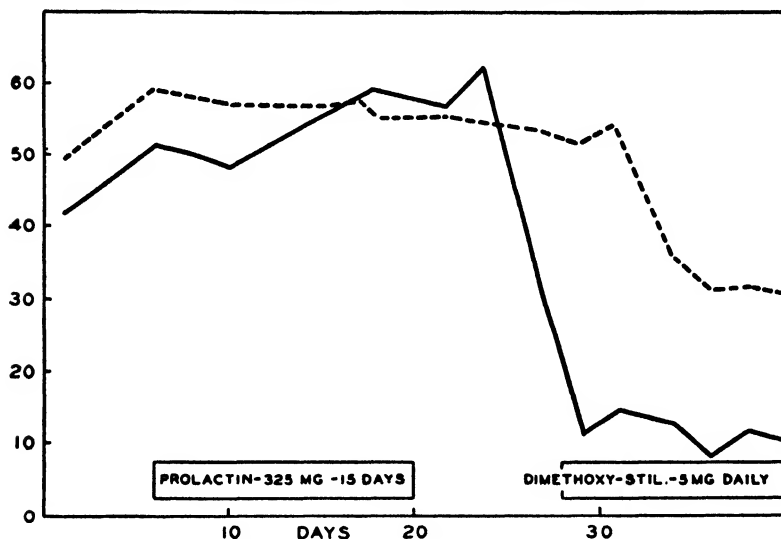
FIG. 23. Failure of prolactin to cause a decline of acid phosphatase in prostatic cancer with subsequent decrease following intramuscular injection of dimethoxy stilbestrol, 5 mg. daily.

From the available evidence, particularly from the demonstration of a considerable content of acid phosphatase in both of the common cancers of the human prostate—those composed of tiny glands and also the undifferentiated sheets of malignant cells, the concept evolved (41, 42) that prostatic cancer was essentially rather mature tissue. It was realized that the presence of large amounts of acid phosphatase in the prostate was a secondary sex characteristic of a chemical nature and that dependent male sex characteristics underwent atrophy on androgenic inactivation

so that some work was undertaken on cancer of the prostate. Fortunately this treatment of the problem yielded rigorous proof.

The phosphatases of serum were used as objective indicators of the activity of androgen and the depression of metastatic prostatic carcinoma by anti-androgenic measures.   In brief, testosterone esters caused a worsening of the disease accompanied by a rise in acid phosphatase activity.   On the other hand surgical excision of the testes or estrogen (Fig. 23) administration (41) or fever (44) often caused a prompt inhibition of the neoplastic condition which was reflected in the phosphatase values.   Estrogens were thus the first agents of known chemical constitution, aside from radioactive materials, which ameliorated carcinomatosis in man or animals and were the first substances known which, when taken by mouth, influenced cancer beneficially.

The advantages of studying the course of carcinoma by assay of the phosphatase enzymes of serum were obvious.   The method was easy and the results were exact and prompt; often within 2 days there was reflected in the serum stimulation or depression of a tumor by modification of the internal environment of the host by a given agent—a highly useful tool for cancer research.

In disseminated prostatic cancers which are favorable from the standpoint of androgen control, acid phosphatase promptly falls and it has remained within the limits of normal for more than 6 years (72) in certain cases, showing that the tumor has been profoundly depressed to say the least.   In these favorably responding cases which have osteoblastic metastasis the alkaline phosphatase of serum usually becomes elevated rapidly above the previous high values and returns to normal in about one to three months; regression of the tumor in some way effects increased osteoblastic proliferation.

Fergusson (67) obtained values of acid phosphatase of 8 to 280 units per gram in 4 malignant prostates and observed that they fell to low values of 1 to 20 units after estrogen therapy.

A variety of response to androgen control results.   In a small percentage of patients these measures have no effect on the enzymes, the cancer or anything else.   In others, the high alka-
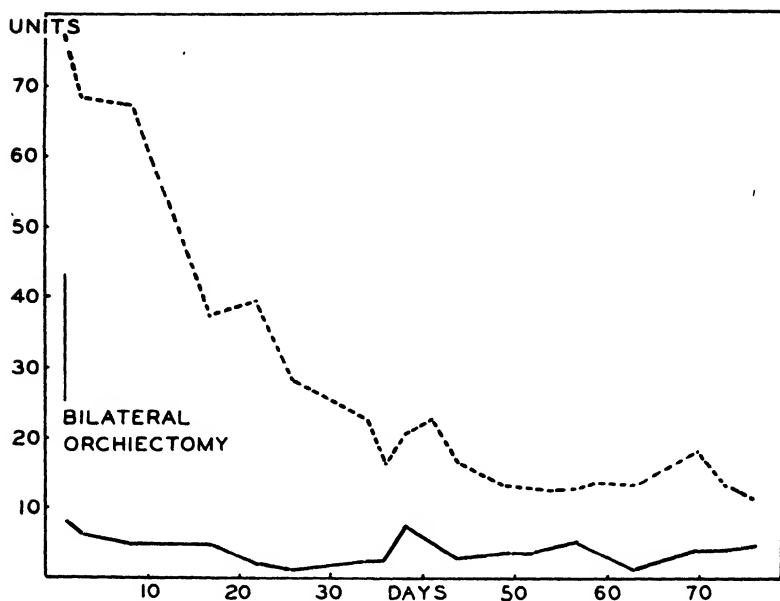
FIG. 24.   Precipitous decrease of alkaline phosphatase of serum following bilateral orchiectomy in prostatic cancer in a man with paraplegia as a result of extra-dural metastasis from prostatic cancer (E.DeB.).   Alkaline phosphatase (· · · · · · ·).   Acid phosphatase (————).

line phosphatase promptly falls to normal without the usual pre-liminary rise (Fig. 24).   In certain cases there are the usual
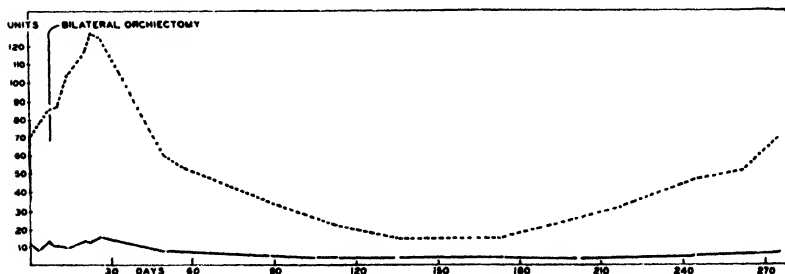
FIG. 25.   Remission in cancer of prostate induced by orchiectomy with subsequent relapse (A.Y.).   Alkaline phosphatase (· · · · · · ·); Acid phos-phatase (————).

signs of remission, but a subsequent relapse (Fig. 25) occurs which is reflected in increased phosphatase values.

In the cases of clinical failure from endocrine treatment in man the cancerous epithelium at times becomes independent of androgen (Fig. 26) since it retains its secretory values in the absence of testis and adrenal (75). In this situation the prostatic epithelium shifts from its category of a dependent struc-

FIG. 26.   Prostatic cancer in a man (F.A.) 116 days after bilateral adrenalectomy and 19 months after orchiectomy.   The epithelium is now self-sustaining in the absence of androgen.   The nuclei are in the middle of the cell, whereas in normal prostatic epithelium they are located basally. × 850.

ture to a self-sustaining one as mentioned at the beginning of this paper.

The close relationship of androgenic hormones and certain enzymes in malignant disease therefore became clearly apparent and chemotherapy was provided which has been of considerable duration—in some cases lasting more than 6 years.

SUMMARY

Observations reported for the first time in this paper include:

1. In dogs systemic illness frequently produces partial or complete inhibition of the pituitary while the testis remains responsive to gonadotrophin.

2. The primary inhibition of estrogen on prostatic function occurs because of deficient production of pituitary gonadotrophin resulting in decreased formation of androgen.

3. Progesterone and desoxycorticosterone do not cause pituitary inhibition.

4. Androgen is not absorbed from pellets at a uniform rate.

5. With respect to the prostate estrogen does not cause complete neutralization of injected androgen.

6. The prostate of dogs is a dual structure consisting of two functionally different areas, the dorsal and ventral segments. Both segments respond to androgen by producing tall epithelium. The dorsal segment becomes transformed to squamous epithelium by estrogen but the ventral segment does not.

When estrogen and androgen are administered the epithelium of the ventral segment remains columnar while that of the dorsal segment becomes squamous. Estrogen injected without androgen produces squamous metaplasia in the dorsal segment and the ventral segment becomes atrophic.

7. Secretory processes of the prostate include both a washing out of preformed constituents and also an active secretion.

8. In certain prostatic glands of dogs fatigue may be demonstrated—a diminished response to a standard stimulus. Fatigue is recognizable as yet only in decreased volume of secretion. More actively secretory glands do not become fatigued in this way.

CONCLUSION

Some elementary theory of prostatic activity has been considered from an experimental basis. The prostatic secretion has been discussed as it occurs in the gland and also as it is secreted in the semen, into the urine and into the blood.

This work has been made possible by the assistance of a great

number of students and friends to whom I am profoundly indebted.

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THE BALLISTOCARDIOGRAPH—AN INSTRUMENT FOR CLINICAL RESEARCH AND FOR ROUTINE CLINICAL DIAGNOSIS[1]

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INTRODUCTION

THE phenomenon which is the subject of this Harvey Lecture has been known for years in certain cases, and every clinician is familiar with it. In many cases of aortic regurgitation and in a few of hyperthyroidism, the bed is shaken by each beat of the heart. This movement of the body, imparted by the movement of the blood within it, is the subject of my lecture tonight.

As soon as this movement was magnified and recorded it became evident that it was not confined to a few pathological conditions; it occurs in every person under all circumstances; but normally it is too small to be seen with the naked eye. Since it is caused by movements of the blood dependent in turn on the strength of the heart's contractions, we are at once challenged to interpret these movements of the body, or the forces which bring them about, in terms of cardiac function. The evolution of medical thought made the time propitious for such an investigation, and I must digress for a moment to explain the conceptions that I found so compelling.

Clinicians have differed widely in their approach to medical problems. In the last century the approach was descriptive, the symptoms of various diseases were described, orderly arrangement of data, that is, classification, was begun. To clinicians with interests such as these, heart disease was a collection of symptoms; dyspnea on exertion, precordial pain, irregularity of the pulse and the like; and to compare the symptoms of one type of heart

[1] Lecture delivered January 16, 1947.

disease with those of another was a major interest. To find a collection of symptoms worthy of a name was the acme of ambition. A friend once told me his great ambition was to discover a syndrome which would bear his name, he belonged to the school of medical thought I have been describing.

This descriptive school was followed by the great pathological school of which Osler was the leader in the United States; and when I was in medical school most chairs of medicine in America were filled by persons who had had long experience in gross pathology. Under the influence of this school there was great interest in betting, during life, on what the autopsy would show after death, and this is still a major interest with some doctors. To such, heart disease is thought of chiefly in terms of organic lesions, as stenosis of the mitral valve, obstruction of the coronary arteries, anterior or posterior infarction of the left ventricle, and the like. Doubtless some of you now present belong to this school, greatly interested in diagnosing the lesions to be found at necropsy.

I mention these schools of medical thought for the purpose of calling your attention to a new school which is arising, the school to which I, and most of my contemporaries belong. It might well be called the Physiological school, provided the term is expanded to include Biochemistry and Immunology. The basic conception is that diseases are processes gone wrong. We are no longer satisfied to think of heart disease in terms of its symptoms, its physical signs, and the lesions found at necropsy. Admitting the importance of all this, for in the perfect conception all the facts will be brought into proper relation, this new school finds its chief concern with questions such as these: what is normal cardiac function and how is this function affected by disease? And so, the heart being a pump, we are vitally concerned with how it is performing its function of pumping the blood, whether it is working well or badly, whether it is weak or strong, words often bandied about by clinicians of the older schools but representing only inferences based on evidence of a most doubtful character.

The chief purpose of my investigations, therefore, has been a

very simple one. I want to be able to answer the question whether a certain heart is strong or weak, and how well it is performing its function of pumping the blood. Interested both in disease and in the care of patients, I was not long content with the difficult and time-consuming procedures for estimating cardiac output available when my work started, though I tried my hand at most of them. The approach finally selected as offering the best chance of developing a method which met the clinical requirements of simplicity, rapidity, and ease of operation was by the ballistocardiogram, i.e., the record of the movements imparted to the body by movement of the blood, or of the forces generated when these movements are restrained.

HISTORY

Now I must emphasize that these movements of the body excited interest long before my entrance into the field. In 1877 a Scot, J. W. Gordon (1), suspended a bed from the ceiling on ropes and obtained a record of its motion synchronous with the heart beat. In 1905, Yandell Henderson (2) built an elaborate suspended table and published a few records, realizing that they were related to the cardiac output. In 1913 a New York clinician, Thomas Satterthwaite (3), obtained a fairly good ballistocardiogram from a patient sitting on spring scales. In 1922 two Englishmen, Heald and Tucker (4), obtained records from a suspended platform but the electrical method they used did not record the direction of the deflection so their records cannot be compared with ours. In 1928 the German geophysicist Angenheister (5) published a few records obtained when normal subjects lay on a table beside his seismograph. Finally in 1933, a Swedish physiologist, Abramson (6), published good records obtained with a beautifully constructed chair, and proposed a formula relating the movements to the cardiac output. I fear that this formula is not valid, but it was the first attempt in this direction and I have always regretted that Abramson was unable to continue his experiments.

These instruments belong to two groups, Henderson's table was free to move, or restrained very little, and recently Nickerson and Curtis (7) have constructed instruments of this type. In contrast, in Abramson's chair and in our own instruments, the movement is restrained by a powerful spring, so that it is at a minimum.

The name of Otto Frank is always associated with the principle that the vibration frequency of recording apparatus must be kept far away from the frequency to be recorded if the record is to be a true one. In designing our apparatus we aimed to get the natural vibration frequency well above the frequency we wished to record, but we encountered difficulties in its construction. Our table had to be extremely rigid and strong enough to support the body weight. Weighted with 150 pounds of iron bars our instruments have a natural vibration frequency between 10 and 15 per second, and this is not as high as it should be according to Frank's theory. Undoubtedly ballistocardiograms with a more rapid frequency could be constructed, but to do so would require such elaborate and expensive apparatus that it would defeat our purpose to obtain a simple and practical device, easily reproduced. Therefore our instrument is not a perfect one.

But the alternative is not without its difficulties also. Seeking a frequency below that to be recorded, Nickerson and Curtis (7) adjust their apparatus to secure a frequency to one per second for each subject; and they employ additional damping. Under these conditions if a record is taken during normal breathing the cardiac complexes are overwhelmed by impacts from the respiratory movements. This is not the case on our apparatus. So to get readable ballistocardiograms on Nickerson and Curtis apparatus the subjects must hold their breath. But if the breath is held with the lungs inflated the record is much larger than if it is held when the lungs are deflated, due to increase in cardiac output as the stretched lungs reduce thoracic pressure and aid cardiac filling. So the size of the ballistocardiogram varies with the position of the chest and I foresee difficulty making untrained subjects hold their breath in the same place. In this procedure a

theoretical advantage has been secured at the price of several practical disadvantages.

But despite these differences of aim and construction the form of the published records secured on healthy persons by the two methods is so very similar that I am not certain whether there is a real difference between them or not.   There is ample evidence that cardiac output can be estimated by either method but probably not with great accuracy in either case.   And it is sobering to recall that we will always be confronted with the problem of recording forces generated deep within the body by apparatus placed outside it.   The body tissues are heavily damped and if struck from without a vibration with a frequency of about six per second is set up.   So the forces we record may well be distorted by their passage through the body, a type of error not to be overcome by perfection of apparatus placed outside it.   For this reason, I doubt if any type of ballistocardiograph gives a completely true account of the forces which originate within the body, and I shall cling to the simplest and most practical type, and learn what I can from it.

Types of Ballistocardiograms.   When speaking about the design and construction of our apparatus it is a pleasure to record my indebtedness to A. J. Rawson, the engineer in charge of the Johnson Foundation shop.   Rawson constructed all my apparatus and his contribution to their design was greater than my own.

We have built ballistocardiographs in many forms: as a table (8, 9), a chair, a platform (10) and a tilt table; and so we can obtain records with the subject lying, sitting or standing.   The table type is by far the most generally useful for clinical work, for many of the sick cannot sit or stand without muscular tremor sufficient to interfere seriously with the record of the cardiac impacts.

Advantages.   The advantages of the method are many.   Taking the records is a simple matter, and from the viewpoint of the patient the method is ideal for he has nothing to do but lie relaxed on the table, or sit or stand normally.

*Disadvantages.*   As any movement made by the subject affects

the record, artifacts are frequent. Records cannot be secured during exercise. Tremors may ruin the record. If dyspnea is severe enough impacts from the respiratory movements appear in the records and may ruin them. Therefore, satisfactory records cannot be made in all cases, but in the horizontal position the proportion of failures is very small indeed.

THE RECORD AND ITS GENESIS

Normal Form of the Record. The records of healthy persons look much the same. At the beginning of electrocardiographic systole the body moves headward a little, the H wave (8). Then, at or shortly after ejection starts, there is a sharp footward movement, the I wave, followed by a headward movement, the J wave, which is the most prominent feature of the normal record. The footward movement K is the last of the major normal defections. After-vibrations follow during diastole, the second commonly higher than the first, and the ripple may or may not last until the following systole. These after-vibrations vary considerably from subject to subject being almost absent in some. A normal record is shown in fig. 1.

The amplitude of the complexes varies with respiration, increasing during inspiration, decreasing during expiration. This is in accord with expectations, as physiologists have long known that the heart is better filled during inspiration which sucks blood as well as air into the chest (14). As you would expect the amplitude varies with the size of the subject. It also varies with age, decreasing in size in the older age groups. Exercise and other agents known to increase cardiac output are followed by impacts greatly increased in size (8).

Genesis of the Ballistocardiogram. I hope this account of the normal ballistocardiogram will stimulate your curiosity as to what is making the body move. Mathematical analysis of the forces involved is a problem in calculus, the basic equations being Newton's laws of motion (8, 10). But I think that most of you will get a clearer idea of the genesis of these movements if I abandon any thought of a mathematical approach at this time
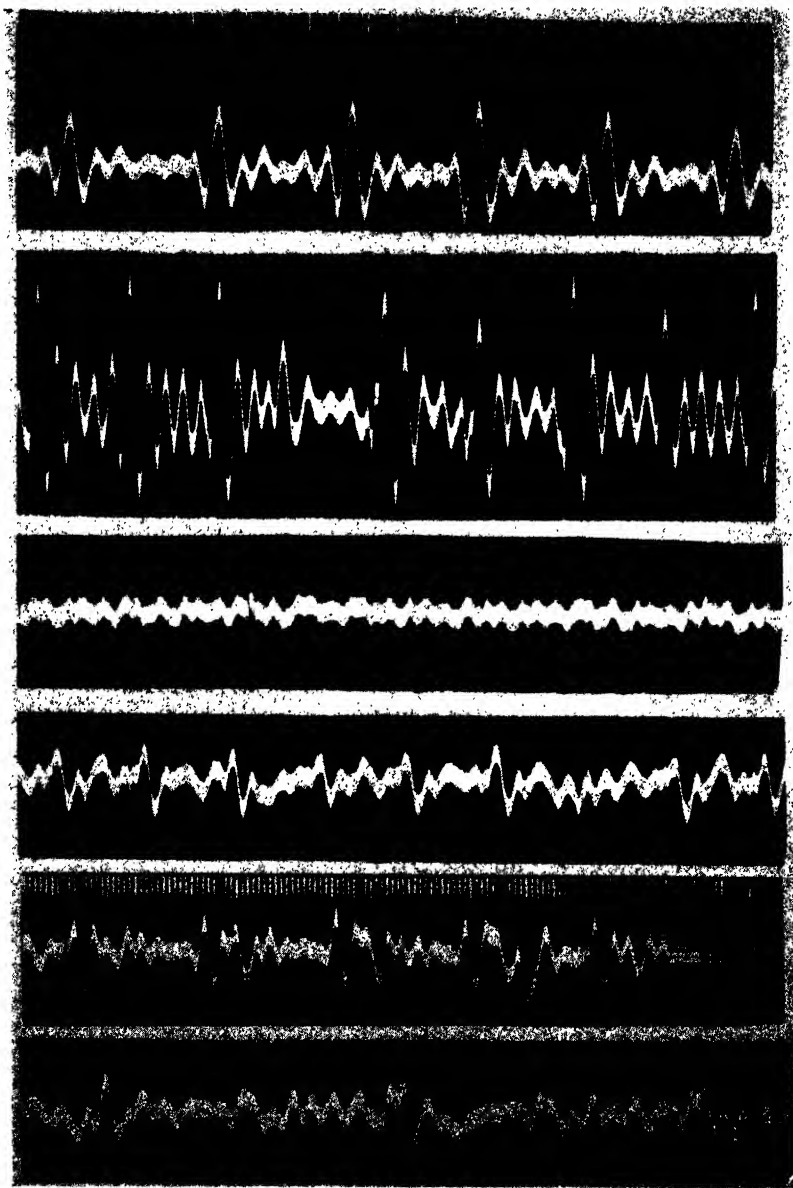
and draw some simple analogies.   I realize well that they greatly oversimplify the problem and neglect details about which opinions differ (13).

Figure 2 shows a syringe (the heart) connected to a tube curved to resemble the aorta; and having a short ascending and a long descending limb; and the cartoon at the top will remind you of what happens when you are standing in a trolley car. Similarly it is the starting and stopping of the blood which causes the ballistocardiogram; if the blood flowed steadily around the body there would be no ballistocardiogram.

Let us now consider the physiological phenomena in the light of

Fig. 1.   Normal and abnormal ballistocardiograms.   The time record at the top applies to all records, the longest interval is one second.   All records were made after a fifteen-minute rest in the horizontal position.   Headward is up.   The reproduction is actual size.   *Top*—Dr. T., age 25, 6 ft. 2 in., 180 lbs., a normal subject.   This record is normal.   *Second*—W.B., age 68, 5 ft. 6 in., 111 lbs.   Hyperthyroidism.   Basal Metabolic Rate + 78.   The cardiac output is calculated to be 148% above average normal.   An extra systole, moving less blood than the other systoles, is seen as the fourth beat. Most complexes are normal in form so the myocardium is judged to be in good condition.   *Third*—E.K., age 51, 5 ft., 119 lbs., B.P. 178/82.   Hypertension and severe angina pectoris.   The systolic impacts are so small it is hard to identify them with certainty.   The record indicates a very severe myocardial abnormality.   *Fourth*—L.S., age 64, 4 ft. 9 in., 123 lbs., B.P. 195/85.   Hyperthyroid, B.M.R. + 29.   This record is very abnormal in form. No normal complexes are seen.   The I wave is absent or greatly diminished in size, the J wave is low in the smaller complexes.   K is deep, and the J K downstroke the most prominent feature of the record.   The record indicates a severe abnormality of myocardial function doubtless due to thyroid heart disease.   *Fifth*—F.B., age 60, 5 ft., 145 lbs., B.P. 108/62.   X-ray shows an aneurism of the left ventricle.   Simultaneous electrocardiogram and ballistocardiogram show that the most prominent upward wave is H.   The J wave is very small and is either notched or flattened.   The presence of the aneurism causes the impact from the left side to lag.   *Sixth*—P.K., age 53, 5 ft, 5 in., 198 lbs., B.P. 225/125.   Essential Hypertension.   Heart not enlarged by orthodiagram.   She feels well and has no complaints.   The record shows one normal complex in each respiratory cycle.   In the others, the I waves are small and J is flattened or notched.   Due to increased resistance in the periphery the impacts from the left side lag behind those of the right.   This heart is showing signs of being unequal to its task.
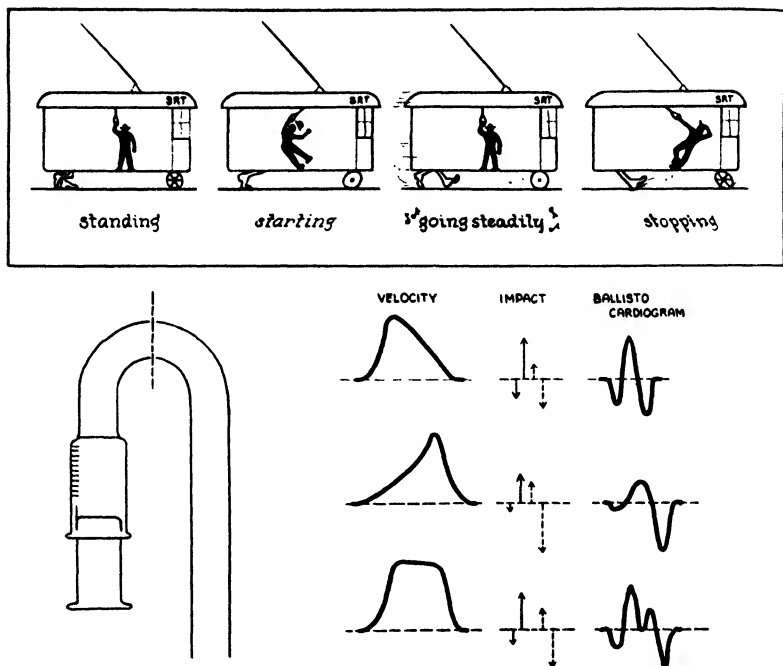
FIG. 2.   Schematic presentation of the essentials of ballistocardiographic theory.   The cartoon above will remind readers concerning certain pertinent aspects of Newton's Laws of Motion.   The syringe and tube represent the heart and aorta.   Under ''Velocity'' have been placed three curves which represent three patterns of ''blood'' ejection velocity during a single systole.   The top curve is believed to be typical of most normal hearts, the second curve is believed to be characteristic of some severely diseased hearts. The third curve is one which will explain a type of ballistocardiogram seen quite frequently in persons with slow heart rates.   Under ''Impact'' are given a series of arrows which roughly represent the resultant of forces developed by the starting (solid lines) and stopping (broken lines) of the blood when its ejection velocity corresponds to the curve adjacent to the left.   Under ''Ballistocardiogram'' are given these curves, roughly derived from the forces by joining the tips of the arrows.   Thus each ballistocardiogram corresponds to the type of ejection velocity curve shown on the same row to the left.   This type of presentation is inaccurate because the force vectors are not given a dimension in time.   But it has proved more useful in aiding non-mathematical readers to grasp the essentials of ballistocardiographic theory than has the more accurate mathematical presentation (10).

fig. 2. The heart contracts and blood is ejected. The blood in the ascending aorta starts headward, the recoil drives the body footward. An instant later the blood in the descending aorta is set in motion footward, the recoil drives the body headward. The solid arrows indicate these two forces; as the mass of blood in the descending aorta exceeds that in the ascending aorta and pulmonary artery, I have made its arrow larger than the other.

When systole draws to a close the blood must decelerate and stop. As the blood in the ascending aorta stops the body will be pushed headward, so I have added the third arrow. An instant later the blood in the descending aorta will slow down, pulling the body feetward, and so the fourth arrow.

Studies by Broemser (11) and by Machella (12) on normal dogs indicate that when the heart is normal maximum blood velocity occurs early in systole and Hamilton (13), deriving the blood velocity curves of man by an indirect method, has come to the same conclusion. The first figure shows the normal velocity curve; its shape indicates that the blood normally accelerates more abruptly than it decelerates, so the starting jerk is greater than the stopping jerk. That is why I have made the first two arrows larger than the last two in the top figure. Connect the tips of the arrows and you have the normal ballistocardiogram.

Now let us suppose that a weak heart is unable to start the blood with such a jerk. Certainly it might save itself the extra work and start the blood more slowly, according to the velocity curve shown in the second curve. Now maximum velocity is attained late in systole, and as systole ends the blood stops with a jerk; so the stopping impacts are greater than the starting ones, and the impact form has changed. In comparison with the normal curve shown above I is shallow, J low and retarded, and K deep. You must keep this change in mind, for I have encountered records of this type frequently among persons with severe heart disease; an example is shown in fig. 1.

In the bottom figure a flat-topped velocity curve has been drawn, and the interest lies in the fact that the corresponding impact form is seen frequently in healthy persons with slow heart rates. This form is not to be considered abnormal.

Other factors enter into the form of ballistocardiographs. As there are effects from both sides of the heart, any condition in which one side is stronger than the other would make the impacts asynchronous and so distort the normal form. I have obtained many records in which asynchronism of the two sides of the heart is the probable explanation of an abnormality of form; fig. 1 gives an example.

I have simultaneous records of ballistocardiogram, electrocardiogram, and brachial pulse in a patient with varying bundle branch block. Where the electrocardiogram is normal the ballistocardiogram is also normal; when the electrocardiogram is abnormal the ballistocardiogram has a shallow I, a retarded and low J, and a deep K, the form to be expected from theory when the heart has lost its snap and the blood velocity curve rises slowly (8). That a change in the form of the blood velocity curve did indeed account for the change in form of the ballistocardiogram is indicated by the pulse wave, which rises abruptly when the ballistocardiogram is normal, but much more slowly when it is abnormal. Hence I have evidence for the correctness of my theory.

Other important conceptions can also be derived from theory. Since the size of the impact depends on the mass of blood moved and the rate of its starting and stopping, the size of the record will vary with the cardiac output. Despite the complexity of the relationship, for both size and shape of the ballistocardiogram enter into it, this is a highly important conception as the cardiac output methods available heretofore have required so much technical skill and have been so time-consuming and laborious that their use in the clinic has been difficult. The establishment of the ballistocardiograph as a method of measuring cardiac output would make such estimations a routine clinical procedure. So this was one of the first problems we attacked.

It was easy to demonstrate the relation in a general way. Situations known to increase cardiac output, such as exercise, food, adrenalin or hyperthyroidism increase the size of ballistocardiograms markedly (15, 16). Situations known to diminish

cardiac output such as myxedema are accompanied by minute ballistocardiograms (17). So our method gives very reasonable results and we were encouraged to define the relationship between ballistic size and cardiac output more exactly.

It was obvious from the onset that any ballistocardiographic cardiac output method would be empirical. But it is also true that, if enough were known about the blood's velocity and mass, and the physical properties of the human body, the cardiac output could be calculated directly from the ballistocardiogram by the equations of Newton's laws of motion. I believe that we know enough mathematics, but not enough physiology to do it accurately. But it seemed worth while to try with what knowledge we had, and a formula was derived which defined the relationship (8). This formula was *stroke volume* $= 33 \sqrt{(2\int I dt + \int J dt) A \sqrt{c}}$. It can be used only for records normal in form.

We compared results obtained by this formula with those secured by the ethyl iodide cardiac output method, a method with which we had worked for some years (8). When the ballistocardiogram was normal in form the formula gave estimations of cardiac output which agreed with results obtained by the ethyl iodide method almost as well as duplicate estimations by the latter method agreed with one another. When results obtained by one cardiac output method are compared with those secured by another one must always remember that all these methods are crude. Duplicate estimations made by a single method often fail to agree closely, so perfect agreement with results secured by a second method must never be expected.

A little later Cournand, Ranges and Riley (18) compared calculations of cardiac output from the ballistocardiogram with estimations made by the Fick method to which they have made such brilliant contributions. These authors justly criticized the estimation of aortic diameter, A in my formula, and substituted a direct measurement in many cases. In their hands the Fick method has given somewhat higher results than was expected from experience with the older cardiac output procedures, and

accordingly they found the ballistocardiographic estimate to average 18% too low. But if a correction was made for this systematic difference then the agreement was excellent in many cases.

Despite these encouraging results it was apparent from the first that the ballistic cardiac output method would have distinct limitations (8). Since it was both evident from theoretical considerations and verified by experiment that my formula could be used to calculate cardiac output only when the form of the record was normal, it could not be employed in many cases of advanced heart disease (8). And Cournand *et al.* (19) have also demonstrated that the results may be seriously off in extreme shock.

In addition to these limitations I have become dissatisfied with my own formula from the mathematical viewpoint and have convinced myself that it would be improved by the omission of the factor representing aortic diameter, A (20, 21). I am pleased about this because the estimation of aortic diameter was subject to grave errors and these will now be eliminated. Fortunately only the square root of these errors has entered into the results obtained to date. After great delay due to pressure of war duties I am now at work on a formula in which "A" is omitted.

The strength of the ballistic cardiac output method, in addition to the simplicity of its operation, is in its ability to estimate changes. The method is at its best in studying changes in individuals and in determining the difference between any person and the normal average. For some time I have been reporting all cardiac output results in terms of deviations from the average of normal persons; as the basal metabolic rate is usually reported. This plan avoids many of the uncertainties, and results expressed this way serve well enough for most clinical problems.

ACUTE EXPERIMENTS

Acute Experiments. The ballistocardiogram is ideal for acute experiments. It has proved very valuable in assessing the action of drugs, both old (25) and new (31, 32), in ascertaining the effects of hemorrhage (22) and intravenous injections (23, 24),

and in following the course of disease, and the effect of treatment upon it (25). I could greatly extend this list.

THE RESTING CIRCULATION IN CLINICAL CONDITIONS

In the task of searching the clinical field for abnormalities I have been fortunate in having able assistants. As their names appear with mine in the bibliography I will not enumerate them here. Without their aid the work would never have been accomplished.

The number of patients tested is now in the thousands. So I have a right to present my data not only from the theoretical viewpoint; i.e., as a method for estimating cardiac output and abnormalities of cardiac function, but also from the empirical viewpoint. Indeed, as our theoretical knowledge is still so limited, I am inclined to believe that the latter is the more important method of approach. Let us, therefore, concentrate on the empirical study and ask ourselves under what conditions abnormal ballistocardiograms are encountered in the clinic and what are the after-histories of those whose records are abnormal. Let us start by dividing the human race into four categories: (1) Those with normal records, (2) Those with records which are of the normal form but too large, (3) Those with records of the normal form but too small, (4) Those with records abnormal in form. For convenience of expression I have coined the words hyper and hypokinemia to express the second and third groups. I have come to use these terms more and more in their literal sense, as increased or decreased movement of the blood, the genesis of ballistocardiograms which are too big or too small, a function usually associated with corresponding changes of cardiac output, but not necessarily so in all circumstances.

Many of the abnormalities we encountered in clinical conditions were to be anticipated from the results obtained by older cardiac output methods. Because these old methods were so laborious the number of cases tested was necessarily small. The contribution of the ballistocardiograph has been to extend this work widely, and in large measure to confirm it. So I have con-

fidence that we now know the deviations from the normal to be expected in many conditions of disease. The ballistocardiogram *provides means of detecting* these abnormalities readily, so one is in position to employ it as an aid in the diagnosis of such conditions.

However, before I discuss the use of the ballistocardiogram in diagnosis I must digress for a moment to be sure you clearly understand my viewpoint. I have little interest in using ballistocardiograms to assist in diagnosing during life the lesions to be found at necropsy and you must not think that the instrument was designed for the purpose of diagnosing cardiac infarction, coronary sclerosis, hyperthyroidism, and the like. It may indeed give help in making such diagnoses, but its chief function I regard as both different and more important. If a lesion be present, the important question is; how does it affect the heart's ability to perform its business of pumping the blood. And so clinicians of the physiological school see in cardiac function itself the fact of first importance to the patient and hence to his doctor. They believe that the estimation of cardiac function will measure the severity of his affliction, and be our best guide to the effect of treatment upon it. So we study cardiac function primarily for its own interest, and only secondarily because its estimation may point the way to some structural lesion, or aid the doctor in giving a name to the disease.

Subjects with Normal Records. Before the abnormal can be identified the normal must be known. Schroeder and I (15) tested two hundred healthy persons in 1938, and for the eight years following I tested a majority of the second year medical class as a part of the course in Pharmacology. Healthy persons acting as subjects for experiments have formed another large group. So my experience with ballistocardiograms obtained in the healthy includes well over one thousand records.

The great majority of hospital patients sick from any cause have normal records also. While I have never calculated it, I should guess that about eighty per cent of all my records were altogether normal.

Subjects with Hyperkinemia. I have omitted from this group cases of aortic regurgitation, all of whom have hyperkinemia but who differ from the others because a portion of the heart's output runs back into it again in diastole, so they do not have a circulation through their periphery which corresponds to their cardiac output. The record of a typical case with hyperkinemia is shown in fig. 1.

Hyperkinemia (16) is encountered in a small percentage of healthy people and this is to be expected. In any method the errors will occasionally summate in one direction to throw the result off. The frequency of hyperkinemia in healthy persons is but little above the figure to be expected from the laws of chance alone. Also, emotion can cause a marked hyperkinemia, and one must decide whether the abnormality was primarily emotional or due to some underlying pathological process. This decision is not always easy. I, for one, do not lightly dismiss the emotions as a cause of ill health, and if the ballistocardiogram proved to be a test of emotion it would make me very happy.

Cases of hyperthyroidism show hyperkinemia with very few exceptions. I have obtained this result on several hundred cases. On the average, the percentage increase in circulation and in basal metabolic rate are equal, as was found by some of the older cardiac output methods. When increased metabolism is found in the absence of hyperkinemia, cardiac abnormality is usually manifest, and, if not obvious, it is to be suspected. I believe that the ballistocardiogram is of real value in evaluating cases of hyperthyroidism.

Patients with extreme emaciation usually show hyperkinemia if the circulation is assessed in terms of their actual weight. But in terms of what they should weigh, their ideal weight, their circulation is normal. So the abnormality is largely a matter of definition. But it is interesting that, while basal metabolic rate diminishes as weight is lost, the circulation does not accompany it downward.

Ballistocardiograms are abnormally large in patients with abnormal intercirculatory communications such as patent ductus

arteriosus and arterio-venous aneurisms. Again this was to be expected fom the older cardiac output studies and the increased circulation may be thought of as an attempt to maintain blood supply to the vital organs in the face of an abnormality tending to draw blood away from them. After closure of the communication by operation the circulation returns to normal in these cases.

In anemia evidence of increased circulation is found often but by no means always.

I have had little opportunity to study infectious diseases. In fever, induced by vaccines or foreign protein increased circulation has been found. Doubtless the same increase would be found early in the febrile diseases. In the later stage of the prolonged febrile diseases the circulation was within normal limits in the few cases I tested. After the temperature first returned to normal the circulation was often subnormal and it often remained so for some days of convalescence.

Hyperkinemia is found quite frequently in chronic pulmonary disease, as it is after pneumonectomy. This finding has long puzzled me, for I see neither reason nor explanation for it.

In addition to the conditions mentioned above, hyperkinemia has been found occasionally in a large number of diverse conditions. I doubt that this has any significance. As emotional states can cause it, it is inevitable that increased circulation due to this cause should be found widespread.

Lastly I find of great interest the fact that hyperkinemia occurs frequently in cases of the type usually called neuro-circulatory asthenia in the United States. In a series of cases so diagnosed in draft board examinations and then referred to me for study, I found this abnormality in a large majority (26). Hyperkinemia without anything found to account for it I think of as Essential Hyperkinemia. Many of these patients are of a type very familiar to experienced clinicians; they are the patients whose appearance and demeanor lead at once to the tentative diagnosis of hyperthyroidism, but the basal metabolic rate is found to be normal. This suggests to me that the appearance associated with hyperthyroidism, so easily recognized at a glance

by experienced clinicians, is determined by the circulatory abnormality rather than by the metabolic rate. If this is true then cases of hyperthyroidism whose circulation diminishes because of heart disease should lack the characteristic appearance. I am inclined to believe that this is often true, for I can think of numerous patients with thyroid heart disease who remained in my ward for some time before it occurred to anyone to estimate the basal metabolic rate, and I wonder if some of my readers may not have had the same experience. Obviously some cases of thyroid heart disease do lack the characteristic appearance of thyrotoxicosis.

Cases with Hypokinemia. Ballistocardiograms which are abnormally small (17) are found frequently in severe heart disease, especially of the coronary type. In this latter group ballistocardiograms often provide the only objective abnormality to be discovered. I will have more to say about this later. A typical record is shown in fig. 1.

About one-third of patients with essential hypertension have subnormal records and this usually occurs in those whose hearts are normal in size. Apparently the human body confronted with hypertension may meet this situation in one of two ways. The circulation may be maintained within normal limits, but to do so requires more work of the heart which hypertrophies in consequence. But there is another course open. The circulation may be allowed to diminish and then hypertension can be maintained without extra cardiac work; so no cardiac hypertrophy develops. One wonders whether it is not the first group who are destined to die from cardiac failure, while the second group may be expected to suffer failure in the periphery as from apoplexy or nephritis, for the peripheral circulation has been sacrificed to spare the heart.

Hypokinemia occurs frequently in endocrine diseases. All cases of myxedema encountered have had tiny ballistocardiograms. This is also true of some elderly diabetics, who, of course may well have been suffering from undiagnosed coronary heart disease. I have seen the same abnormality in Cushing's

syndrome. In Addison's disease where I fully expected to find subnormal ballistocardiograms the majority of my cases have given normal records.

Hypokinemia is often found in convalescence from the severe infectious diseases as has been mentioned.

Finally, subnormal ballistocardiograms are found in persons with symptoms referred to the cardiovascular system but in whom no disease could be demonstrated by the usual clinical methods. From their symptoms these patients also fall into the group called functional heart disease or neuro-circulatory asthenia. I mentioned before that some patients so diagnosed had circulations above normal, and it is becoming more and more obvious that the group is a diverse one (27).

Patients with Ballistocardiograms of Abnormal Form. I am now engaged in the study of this group (28). Three examples are shown in fig. 1.

These abnormalities of form may affect every complex equally but more frequently the form varies with the respiratory cycle. Thus I have many records in which the smallest complex of each respiratory cycle is abnormal in form, every other complex being normal. In other records only the largest complex of each respiratory cycle remains normal.

The shape of these abnormal complexes may also vary regularly with the respiratory cycle, and this is quite common. In contrast, in auricular fibrillation the impact form varies from beat to beat in a completely irregular manner, as one would expect.

So to assess the degree of abnormality by inspecting the record one must have multiple criteria in mind. I first note the proportion of abnormal complexes and then study their shape. It seems evident that the proportion of abnormal complexes bears a direct relation to the severity of the myocardial abnormality but to say which of the many aberrations of form is the most ominous for the patient is beyond my power at present.

In certain cases in which the form is abnormal when lying at rest, if the patient takes a deep breath and holds it the form becomes normal. The explanation of this is of course related to

that of the records in which abnormality is confined to the smaller complexes of the respiratory cycle; some diseased hearts seem able to contract normally only when their filling is most adequate, a finding consistent with Starling's law of the heart.

Grouping all the abnormal types together it has become evident that ballistocardiograms abnormal in form are found in three groups of patients. As one would expect, such abnormalities are found most frequently in patients with manifest organic heart disease; I have 138 instances in my files. In this group the abnormal ballistocardiogram confirms the presence of a myocardial abnormality which would be expected by any clinician from the history and physical examination.

Records abnormal in form are also found with great frequency in cases of hypertension; I have 85 such records to date. Some of these patients had, some did not have heart disease manifested by the usual methods of examination. I find this very interesting because the heart, having to contract with unusual force to eject blood at normal velocity against the increased pressure might save itself work by contracting with less snap. The great majority of early cases of hypertension give perfectly normal ballistocardiograms, so that in these the heart seems adequate for its task. When the record becomes abnormal I interpret it as indicating that the heart is showing the first signs of being unequal to its task.

Abnormal forms have also been found frequently in patients over 50 years of age soon after they had undergone severe surgical operations (9). In many of these cases normality was regained during convalescence.

Finally major abnormalities of form have been encountered in 24 patients without hypertension and without other evidence of cardiac disease despite careful study by the usual clinical methods. Naturally I believe that these abnormal ballistocardiograms demonstrated abnormal cardiac function in these patients. If this is true one is forced to conclude that the ballistocardiogram gives evidence of cardiac damage of a type not detected by other clinical methods. I have one case which demonstrates that

this is indeed true (28). In this patient all cardiac studies were negative except the ballistocardiogram which was highly abnormal. Death took place several days later and necropsy showed extensive diffuse coronary sclerosis in addition to the infarct which caused her death.

Before concluding the discussion of records abnormal in form, I must describe a fairly common finding which has always puzzled me. I have a good many records in which ballistic form was highly abnormal when the patient was lying at rest. But when he arose and stood upright, the record increased in amplitude and became normal in form. I have no very satisfactory explanation for this phenomenon, and I am driven, much against my will, to the following position. Under some circumstances myocardial weakness is most apparent when the heart is taking it easy, so to speak. When a slight demand is made on the heart, as by a situation requiring an increase of circulation, the myocardial reserves are called out and the abnormality is overcome for a time. This view does not deny that a still greater demand on the heart may exhaust its reserves and make abnormality detectable, as I have demonstrated in many cases.

FUNCTIONAL TESTS

I have talked hitherto chiefly about the results of tests conducted with the subject lying at complete rest and certainly such tests demonstrate abnormality in many cases. But one has every right to expect that incipient disease would first manifest itself, not at rest, but when the organism was under strain. Since ballistocardiograms are so easy to take, the method can be readily adapted to making tests when the subject is under a stress. So we have been interested in testing patients under two very mild stresses; the first is the upright position and the second after mild exercise.

The Response to Arising. A comparison between the pulse rate, blood pressure and ballistocardiogram at rest, and the values obtained after standing two and one-half minutes, has been made in many hundred persons, and adequate normal standards are

available (29).   Such a test gives information, not so much about the heart as about the vasomotor system which must become active to prevent the blood from gravitating to the feet.

When a normal person arises the average circulation as calculated from the ballistocardiograms, remains the same, although I myself, tested repeatedly over a period of years, have consistently had a smaller circulation when I stood upright and there are many examples of this in the literature.   In many conditions of disease highly abnormal results are obtained.   I had fully expected the circulation to diminish abnormally in disease, and this is found occasionally, especially in cases of advanced heart disease.   But the opposite abnormality, the impacts increasing abnormally on arising, is extremely common in all sorts of conditions of mild ill health.   What we are demonstrating may be characterized as an incoordination of the circulation; that is, a failure to control it properly.   This failure of the circulation to adjust properly to the upright position is perhaps the reason why one is so much happier in bed when one is sick.

This brings me finally to a definition of the abnormality of those cases who have symptoms referable to the circulation without any organic lesion to account for them, the group generally known as neuro-circulatory asthenia in this country.   By means of criteria wholly objective we have demonstrated that the abnormality present in the majority of these cases is an inability to adjust the circulation to the needs of the moment in the normal manner.   They suffer from an incoordination of the circulation, an inability to control it properly and I am hopeful that, now that objective means of assessing the abnormality are at hand, methods of treatment can be more adequately tested and that advance in therapy may be expected to follow.

Finally from this large experience of testing the circulation of the sick in both the vertical and horizontal position, a generalization can be made which, to my mind, is of first importance, especially because it is so little realized by most physicians.   The circulation, when the subject is upright, is under entirely different controls than when the subject is recumbent.   Drugs,

other physiological agents, and conditions of disease may affect the circulation very differently in the two positions. For example, rapid hemorrhage causes either slowing or little change of the pulse rate when the subject is recumbent, but if he is upright the pulse is greatly accelerated. The drug paredrine slows the heart when the subject is recumbent, accelerates it when he is standing. Any description of such effects on the circulation must be made with reference to the position of the subject. I fear there is much confusion already in the literature because of ignorance of this fact.

After Exercise. Another method of imposing a strain on the heart and circulation is by exercise. Unfortunately it is impossible to secure ballistocardiograms during exercise, but they may be obtained as soon as exercise has ceased. Early in my work I secured a series of records on patients before and after ten to twelve trips over the two steps described by Dr. Master (30). A difficulty soon appeared, for some patients with cardiac disease had so much hyperpnea after the exercise that diaphragmatic impacts interfered with interpretation of the cardiac impacts. Nevertheless, I obtained some striking contrasts. In normal persons the impacts increased both in size and rate after exercise, but some patients with cardiac disease, while showing the usual increase of heart rate after exercise, had impacts actually smaller than when they were at rest. Apparently certain types of diseased hearts confronted by the demand for increased output, can respond only by increasing the rate, as if increased output per beat were impossible for them. And so these exercise tests provide another means of detecting cardiac abnormality.

Abnormalities of form may also be brought out by exercising the patient in many instances.

EVALUATION BY AFTER-HISTORIES

The final test of any clinical method is by its utility. When we assembled 200 healthy persons in 1937, 1938 and 1939 to determine the normal standards (15), we realized that their after-

histories might well be decisive in the evaluation of ballistocardio-grams. Therefore the group was picked, as far as possible, from the permanent hospital population and from the families and friends of the authors. From eight to ten years have now elapsed since these tests were made. Fifty-five persons were over fifty years of age when first tested, and we have obtained information about the present state of health of forty-seven (28). Fig. 3 summarizes this data.

Reexamination of the records obtained eight years ago in these forty-seven cases showed that twelve had unusually small impacts, and so their cardiac output, as calculated by our origi-nal "area" formula, was subnormal. Of these twelve, six have developed coronary heart disease of sufficient severity to lead to their making contact with the hospital, and another has died in congestive failure. Another of the group has also died, but not of heart disease. Therefore about 58 per cent of those who had abnormally small impacts eight years ago have developed serious heart disease during the eight-year period.

Of these persons over fifty years of age thirty-four had im-pacts of normal size and their cardiac output was calculated to be within normal limits. Of these two have died, neither of cardiac disease. One has developed undoubted coronary heart disease. The remaining thirty-one are known to be alive and actively working, none of them has come to the hospital for study, and those who could be reached have denied cardiac disability of any sort. Therefore only 3 per cent are known to have de-veloped serious heart disease in the eight-year period.

The difference in incidence of the development of serious heart disease in these two groups is highly significant (58 per cent in contrast with 3 per cent) and leads to the expectation that we can detect coronary heart disease before the development of the typical clinical picture in many instances. However, it should be noted that in one instance mentioned I failed to detect any abnormality before the development of typical manifestations and this is also true in one other case not included in this series because he was a hospital patient and not in good health when the first test was made.
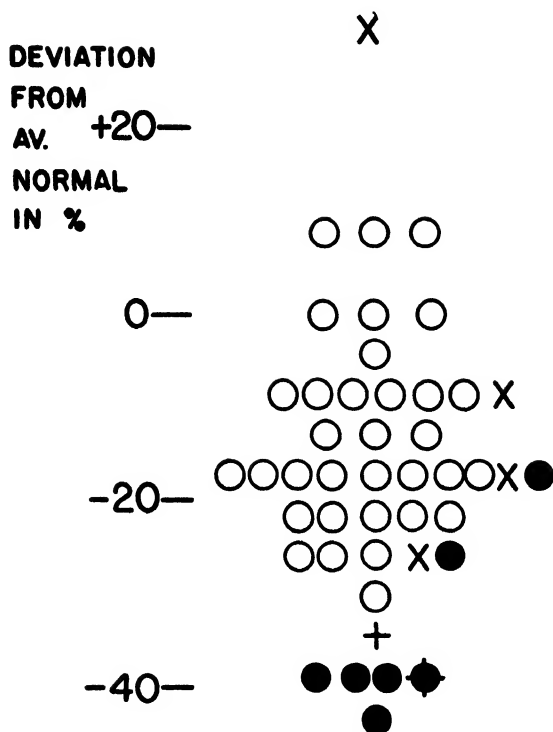
FIG. 3. After-histories of a group over fifty years of age tested as healthy persons during 1937–1939. The symbols have been placed according to the deviation of the cardiac output calculated from ballistocardiograms taken during 1937–1939, from the average of healthy persons between 20 and 40 years of age. The normal limits usually employed are ± 22% from this normal average. The character of the symbols refers to the status of these subjects in 1946.

In addition to the cases mentioned above two of the supposedly healthy persons over fifty years of age gave ballistocardiograms whose form was abnormal.   These could not be included in the percentages given above because their cardiac output could not be estimated.   One of these has had several mild attacks of coronary occlusion during the eight-year period; the other has made no further contact with the hospital.

I am now reviewing the after-histories of those who were between forty and fifty years of age when tested eight to ten years ago, but the data is not yet all in.   However, two of these persons had ballistocardiograms abnormal in form in 1938.   One of these dropped dead at the wheel of his car a few months ago, the other, whose record was far less abnormal, suffered from a mild attack of cardiac infarction in 1940.

It appears, therefore, that a ballistocardiogram either abnormal in form or abnormally small is of serious prognostic import.

I conclude therefore that the investigation of clinical subjects by the ballistocardiogram is an intriguing business, and I am emboldened to suggest that by the same means others also could discover facts well worth knowing about the patients who come to their care.

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THE CAUSES AND CONTROL OF
MAMMARY CANCER IN MICE[1]

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A S YET little information is available regarding the nature of the cellular changes which either precede or are initiated by the transformation from normal to neoplastic tissue. However, with the accumulation of data on the genesis of mammary cancer in mice, it is evident that several causative factors are involved and it is possible that others may be demonstrated in the future. With this in mind, it is not the purpose of this discussion to theorize about the genesis of this type of malignancy but to indicate by experimental data what the inciting causes may be and how mammary cancer in mice may be averted. Any interpretation of data will be advanced only after consideration of the problem as a whole and not on isolated experiments. They are based upon our present information and must be subject to revision as new observations are obtained.

The first studies on mammary cancer in mice were concerned primarily with the characteristics of the inherited susceptibility to the disease. Murray (119, 120) showed that mice with a cancerous ancestry were more likely to develop such tumors than were animals without such a background. Many articles have been published by Slye in which she presented the results secured by crossing various cancerous and non-cancerous lines (these lines did not compare with the inbred strains which are being used at

the present time). In 1926–1927 (139, 140) she advanced a recessive theory to explain the transmission of the inherited susceptibility for all types of cancer in mice. The hypothesis was modified in 1937 (141) and again in 1941 (142). According to her most recent interpretation, mammary cancer in mice would be inherited as a double recessive with the tendency to late malignancy dominant over the tendency to early malignancy.

Although Loeb and his associates (104, 105) did not have access to inbred strains, the results which they obtained from several of their crosses between high and low cancerous strains were quite comparable to those which are being observed at the present time. In addition to the inherited susceptibility, the rôle of the hormones was investigated and Loeb (111) concluded that the initiation of mammary cancer was dependent primarily upon heredity and the functional activity of the sex hormones, expressed either as H (heredity) + S (stimuli) = C (cancer), or H × S = C.

That some maternal or extrachromosomal influence was also involved was indicated in independent work by the Staff of the Jackson Memorial Laboratory (92) and Korteweg (100). These observations resulted from several reciprocal crosses between strains with high and low incidences of spontaneous mammary cancer. In every cross the hybrids with maternal parents from the cancerous strains showed high incidences whereas only a few tumors appeared in the reciprocal hybrids.

Such a maternal influence could be transferred to the offspring by any of the following methods:

1. By way of the cytoplasm or cytoplasmic inheritance.
2. During intra-uterine development.
3. By means of the mother's milk by nursing.

Cytoplasmic transmission (101, 129, 106, 107) of the maternal influence has been suggested as has the possibility of some intra-uterine influence (73) for mammary cancer in mice.

The results of foster nursing were first presented in 1936 (29, reviewed in 44, 49, 136, 146, 13). In this study the young born to females of a cancerous strain were removed soon after birth

and were transferred to lactating females of a low cancerous strain. In an extended series (33), it was found that less than 10 per cent of the fostered mice had mammary cancer whereas the mice which had been nursed by their mothers of the cancerous line had an incidence of approximately 90 per cent. In general, the fostered mice which died cancerous had cancerous offspring although the same applied to some fostered mice which did not develop mammary cancer. Other fostered mice which remained non-cancerous (mammary) had progeny and descendants with a low incidence. These observations demonstrated that some agent was transferred in the milk and that a female of the cancerous strain did not have to be cancerous to do so.

Further work showed the importance of the interval between birth and transfer to a foster mother. If the young were permitted to nurse their mothers of cancerous stocks for a number of hours before they were fostered, little or no reduction in the incidence was noted. By such methods it was found that the agent was present in the milk secreted after the first day (30) and during the entire nursing period (15). The removal of the young from the uteri of females of cancerous stocks was found to be more effective than foster nursing in preventing transmission of the milk agent (7, 16).

As stated above, fostered mice which die non-cancerous usually have progeny and descendants with a low incidence of mammary cancer. Two such lines have been continued for 20 and 30 generations without further foster nursing with only about 1 per cent having developed mammary cancer (45, 49, 54); other sublines descended from the same mothers have given rise to high cancer lines (38, 45). If the descendants of the fostered females with low incidences are nursed by females of high cancerous stocks with the agent, the incidence in these fostered mice approximates that seen in the high cancerous stock (33). Further studies showed that by either feeding or injecting material containing the milk agent, it was possible to produce mammary cancer and thus the distribution and characteristics of the agent could be determined. These data will be considered later.

After the importance of the maternal or extrachromosomal influence (milk agent, 29) was recognized, the rôle of the inherited susceptibility as a causative factor in the development of mammary cancer was relegated to a minor position for several years. It was suggested (128, 130) that some extrachromosomal influence was six to ten times as important as any chromosomal factor and that the tendency to have mammary cancer was not mendelian in nature; more recently data were interpreted to mean that the inherited susceptibility may be of greater consequence than the milk agent in the genesis of mammary cancer in mice (159).

In order to simplify the presentation of the results of various experiments, it seems advisable to enumerate the major inciting factors which have been demonstrated to play a rôle in the genesis of spontaneous mammary cancer in mice. These are (32):

1. The inherited susceptibility.
2. Hormonal stimulation.
3. The mammary tumor milk agent.

Since each factor is nearly completely determining in its effect, it has been asserted (32, 44, 49, 136) that they are of equal importance, or to quote from another (85), "Mammary tumors do not result from any one of these factors or sets of factors, but from the action and interaction of all three, and it would be folly to attempt to say which is the more important."

The virgin females of some cancerous susceptible strains may show a low incidence (31) whereas the virgin females of other strains may show high incidences (5, 55, 86). The difference in the incidences between the virgins of some strains has been demonstrated to be due to the genetic control of the hormonal mechanism (55, 86, 54), termed the inherited hormonal influence (49). This introduces another inherited factor, apparently independent of the inherited susceptibility, which operates in the genesis of mammary cancer in virgin females.

If the young born to females of "low cancerous strains" are nursed by females of stocks with high incidences, no strain or

line has been found which will remain completely free from this type of malignancy. The lowest incidences to be detected in breeding females of various lines of the C57 black stock have been about 10 per cent (3, 36, 39). Comparable incidences have been found in fostered mice of this stock which were maintained as non-breeders (156, 127) although others have data, based on small numbers, which suggest that the females must give birth to young before mammary tumors may result (73, unpublished data). Breeding females of other sub-lines of the C57 black stock may show incidences of 50 per cent (19, 73), 63 per cent (8), and even 76 per cent (83) after they had obtained the milk agent. In some series the progeny of the fostered C57 black mice were found to have approximately the same (83) or a slightly higher incidence (73) than did the mice of the fostered generation. In other experiments, the fostered mice and their progeny gave a low incidence but in one study (36) susceptible mice nursed by such females had a high incidence; it (10) has also been reported that only the mice of the fostered generation would transmit the milk agent. Andervont (3) determined that while the fostered mice of lines of the C57 black and I stocks showed incidences of 14 per cent and 17 per cent respectively, the hybrids produced by mating females of the C57 and males of the I strain gave, when fostered by females of the same high cancer stock, an incidence of 71 per cent.

From these data it is apparent that there are sub-lines of the C57 black strain which have different "susceptibilities" for the development of mammary cancer. Also, if we assume that a line which gives an incidence of 10 per cent (the lowest incidence to be obtained to date in breeding females) to be non-susceptible, then we must accept the possibility that some mammary tumors may result from the action of the milk agent and hormonal stimuli. Such data make it difficult to analyze the genetic make-up of the inherited susceptibility for mammary cancer in mice.

Several experiments have been made on the nature of the inherited susceptibility (31, 32, 3, 4, 6, 42, 126, 127, 46, 8, 47).

Since non-breeding females probably must have, in addition, the inherited hormonal influence (55, 86, 54) before a high incidence may be expected, it seems advisable to use parous females for these investigations. This latter influence may be determined by some genes which are not identical with some which determine the inherited susceptibility (55).

Data collected from a large number of mice in reciprocal crosses between the cancerous A and the low cancerous B (C57 black) stocks, led to the following conclusions regarding the inherited susceptibility for mammary cancer (47):

"No evidence was secured to suggest any intrauterine influence for the development of mammary cancer in mice.

"The incidence in the total number of F_1 hybrids nursed by females of the A stock did not differ significantly from the incidence secured in mice of the cancerous A stock.

"Although the pooled data obtained in all the hybrid generations could be accounted for on the genetic theory that the inherited susceptibility for mammary cancer in this cross was transmitted as a single dominant factor, it becomes evident on analysis of the incidence of cancer in the several subgroups that such a simple interpretation is inadequate and that other factors are probably involved. Detailed analysis shows:

"1. That the incidence of mammary carcinoma in the F_2 hybrids with brown coat color was significantly higher than in their litter mates with black or albino coats.

"2. That not all brown mice became cancerous, nor was the incidence in brown mice as high as in mice of the high cancerous A stock.

"3. The progeny of brown mothers had a higher incidence than did the progeny of black or albino mothers, but not all brown mothers transmitted the inherited susceptibility to their progeny.

"4. The incidence of cancer was significantly greater, in some groups, in the mice born in the third and later litters than in mice born in the earlier litters from the same mothers.

"5. The incidence in the progeny was influenced also by the age at which the mothers developed mammary cancer.

"If some mice of a genetically nonsusceptible strain develop mammary cancer, it is probable that some cancerous hybrid animals are likewise nonsusceptible. Thus, the incidence would not represent the true percentage of susceptible animals and should not serve as the only basis for a genetic interpretation of the data.

"It is concluded that the inherited susceptibility for mammary cancer in mice, as transmitted by mice of the cancerous A stock, probably depends upon multiple genetic factors, one of which may be linked with the gene for brown coat color."

The determination of the number of genes which are transmitted to produce the inherited susceptibility is further complicated by other factors. Mammary cancer is produced by at least three inciting factors and the action and interaction of these possible inciting causes may not be identical under all conditions.

Even though the inherited susceptibility for mammary cancer may determine the sensitivity of the animals to the action of the milk agent, mice with the same genetic make-up still might not have the same incidences if they obtain the milk agent from different cancerous strains. In some crosses between cancerous stocks considerable variation is to be found in the reciprocal hybrids even when they are maintained as breeders (28), in other crosses such a difference is apparent only in the virgin females (126, 55, 159) and not in the breeders (54). Further studies are needed to ascertain whether or not these effects might be mediated through the physiological action of hormonal stimuli as well as perhaps by the genetic susceptibility.

Heston, Deringer, and Andervont (87) have implied that instead of the inherited susceptibility, another set of genetic factors determine the propagation of the milk agent. This theory was based upon the fact that the mice of the C57 black stock were considered to have "100 per cent resistant-strain chromatin" and that all the tumorous females of a back-cross generation to the C57 black stock transmitted the agent whereas only some of the non-tumorous females transmitted the agent while others did not.

If the propagation of the milk agent was controlled by genes which were not the same as those which give rise to the inherited susceptibility, it would follow that the females of the back-cross generation to the resistant (C57 black) stock, although they developed mammary cancer, would not propagate the agent as well as the back-cross animals to the susceptible (C₃H) strain. That is, there should have been segregation between the two sets of genes which determined the inherited susceptibility and the propagation of the milk agent. Since the incidences in the mice fostered by females of these respective groups were 91 per cent and 93 per cent, no segregation was apparent. Non-cancerous females of the C₃H back-cross generation should inherit both sets of factors and yet the incidence in the young which they nursed was only 50 per cent, the same as that observed in the young fostered by females of the C57 back-cross generation. This would suggest that non-cancerous mice, regardless of their genetic constitution, did not propagate the milk agent as effectively as did the cancerous mice based upon the incidences in their fostered progeny. The exact interpretation of these data is impossible with our present knowledge. In another publication Andervont (10) found that the mice of the same sub-line of the C57 black stock would transmit the agent when they were members of the fostered generation. This would corroborate the findings of others that mice of the low cancerous C57 black stock may propagate and transmit the milk agent.

Thus we probably must wait until a strain has been found which will remain non-cancerous after they have obtained the milk agent and perhaps will not transfer the agent before we shall be in a position to understand the various data mentioned above. Obviously, no theory will account for the results in every study.

Lathrop and Loeb (105), Loeb (109, 110), Cori (61, 62), and Murray (121, 122, 124) investigated the effects of ovariectomy upon the genesis of mammary cancer in mice and were in agreement with the general conclusion that the younger the animals were when their ovaries were excised, the lower the incidence

would be in the operated animals.   Later, Woolley, Fekete, and Little (169–171) ovariectomized one-day-old mice of the same strain which Murray had used and found that some gave rise to spontaneous mammary cancer.   The spayed animals showed estrus and evidence of hormonal stimulation of the uteri, mammary glands, etc.   The source of the hormones in the absence of the gonads was found to be the hyperplastic cortex of the adrenals.

An association has been found between the hyperplastic changes in the adrenals of ovariectomized mice of some stocks and the inherited hormonal influence (143–145).   While mammary tumors have arisen only in mice which had the milk agent, animals of the same strain without the agent have had comparable adrenal alterations and resultant secondary sex organ development.

The rôle of the hormones in the genesis of mammary cancer has also been studied by the use of castrated males with ovarian grafts.   Although they failed to develop in the series done by Loeb (110) and Cori (62), in other experiments some tumors did arise (122, 94).   In a recent study (91) the incidence of mammary tumors was as high or higher in the castrated males with transplanted ovaries than was obtained in the virgin females and the average age in the males was six months earlier, only one month later than was observed in the breeding females of the same stock (54).

Lacassagne (102) determined that mammary cancer might be induced in male mice by the injection of estrogenic hormones (subject reviewed in 75, 111, 1) but few tumors have been produced in either females or males of cancerous stocks unless they have the milk agent (103, 40, 76, 134, 135, 137, 58).   Estrogenic induced tumors may arise also in males of low cancerous stocks after they received the milk agent (155).

Either the incidence of mammary cancer in mice is higher in parous than in non-parous females of the same strain or, if the incidences in the two groups are approximately the same, those subjected to the increased hormonal stimulation associated with

pregnancy and lactation have an earlier average cancer age. Extensive reviews on this problem are available (75, 111, 1, and 146).

Murray (125), Bittner (25, 26) and Bittner and Murray (56) compared the breeding behavior of females of several high cancerous stocks and could not find any significant difference between the mice which died either cancerous or non-cancerous.

In a report on one of these strains, Jones (93) observed that in her line of the A stock the incidence of mammary cancer was directly proportional to the number of litters born. Such findings are not characteristic of all strains (132, 114, 115, 24, 73). Jones studied two groups of females; some were separated from the males after they had given birth to either one or two litters while others had a longer breeding period with some having as many as ten litters. The females which had only one litter had an incidence of 12.6 per cent; two litters, 24.4 per cent; and three or more litters (average, 4.2), an incidence of 49 per cent. The incidence for the total number was 24.6 per cent at an average cancer age of 14.8 months. With the increase in the number of litters, there was a tendency for the females to develop mammary cancer at earlier average cancer ages.

While we do not have data available as yet to state what the incidence may be in breeding females of the A strain which were permitted to have only one litter, our observations on the incidences and average cancer ages for the females which were permitted to have as many litters as they would before they developed mammary cancer are quite different than were found by Jones (93). The tabulation of nearly 700 breeding females showed that only one animal had a total of eight litters (average 4 per female). The incidence of tumors for the total was in excess of 90 per cent and the average cancer age about 10 months. Those which had less than three litters had an earlier average cancer age than did the females which had four or more litters and there was no significant difference in their incidences. Comparable findings were obtained when the incidence in the cancerous C_3H stock was tabulated based on the breeding behavior of the females.

It would be expected that little similarity might result since the lines of the A strain which were compared had different incidences (nearly 70 per cent) and average tumor ages (5 months).

Although Murray (123) was unable to find any variation in the incidence of tumors among mice of the dilute brown cancerous stock which nursed none or all of their litters, Bagg (17–21) reported that rapid breeding and the prevention of nursing, now usually referred to as force breeding, has some effect on increasing the incidence of mammary cancer in mice of the low cancerous C57 black stock. Little and Pearsons (108) and Fekete (71) were unable to confirm these results by subjecting mice of other sublines of the same stock to the test.

After mice of the C57 black stock have been nursed by females with the milk agent, incidences from 10 per cent (15, 36) to 76 per cent (83) have been observed and the highest was found not in force breeders but in a group of females which was permitted to nurse their young. In view of these recent observations, it is probably of significance that in Bagg's earlier studies some of the mice which developed mammary cancer had been raised by foster mothers from whom they might have received the milk agent (44). In an extension of his investigations, Bagg (19) found that in one line of the C57 stock, mammary tumors appeared only in some of the mice which had received the agent by nursing.

Fekete (71) reported on the effects of three "functional tests" upon the development of mammary cancer in mice of the C57 black and the cancerous dilute brown stocks. Tumors did not appear in mice of the former strain in any group. Females of the dilute brown stock which had lactation prolonged by the addition of young mice at regular intervals showed an incidence of 56 per cent; those which gave birth to and nursed their young in the breeding pen had an incidence of 72 per cent; whereas the females which nursed their young for only one day before they were returned to the breeding pen (comparable to force ' breeding) gave an incidence of 87 per cent. No significant dif-

ference was noted in the average ages (413 to 425 days) at which the tumors appeared in the mice of the various groups but those which had fewer pregnancies followed by prolonged lactation had the lowest incidence. It was considered (73) that force breeding had not increased the incidence of mammary cancer but comparisons were not made with females which bred and nursed in the usual way.

The influence of complete blockage of the nipples on one side in mice which were permitted to lactate and nursed their young via the unaltered nipples was studied by Fekete and Green (72). As with force breeding, this treatment did not result in the development of mammary cancer in mice of the low cancerous C57 black stock. In mice of the cancerous dilute brown strain, more tumors appeared on the blocked than on the other side and the authors concluded ''that blockage of the mammary ducts with resulting milk stagnation was influential in determining the site and the time at which mammary tumors appeared in a genetically susceptible strain, but did not itself cause tumors, since it was unable to overcome the hereditary resistance in animals of a strain in which such tumor normally did not develop.'' The average age of the mice at the time the tumors developed on the blocked side was 11.2 months as contrasted with 12.7 months on the unblocked side. These average ages are earlier than those found in force bred mice of the same strain (71) but the incidence, 82 per cent, was lower although several tumors are included which were discovered only after histological examination of the glands. The data for normal breeding females were not presented for comparison.

To obtain other information upon the rôle of force breeding with the prevention of nursing upon the genesis of mammary cancer in mice, females of the cancerous C_3H strain were subjected to this method of breeding. The mice were kept in the breeding pen (5 females to 1 male) and the young were removed within a short time after birth. Data were also obtained on females of the same strain which were usually removed from the breeding pen before their young were born and which were per-

mitted to nurse their offspring either until weaning age or until all the mice in the litter had died, after which the females were returned to the home pen. These are referred to as "normal breeders."

During the past four years the average age at which mammary cancer has developed in normal breeding females of this strain has been 273 days and the incidence has been 95 per cent (54). Observations have been completed upon 54 normal breeders and 29 force breeders which were born during the same period. The normal breeders had from one to six litters, average 3.9, whereas the force breeders cast from 5 to 8 litters, average 6.3. In the former, the average cancer age increased with the number of litters born to the females but in the force breeders there was little variation in the average cancer age of those which had from 5 to 8 litters, however the numbers are too small to have significance. Whereas the normal breeders had an incidence of 94.4 per cent at an average age of 272 days, the mice subjected to force breeding had an incidence of 82.8 per cent and an average cancer age of 310 days. The non-cancerous mice of the former group averaged 269 days at death but the non-cancerous force breeders lived to an average age of 395 days.

The only other reference which we could find in which force and normal breeders were compared was given by Bagg (21). In sub-line Y of the C57 black stock, the females continued as force breeders had given an incidence of 42 per cent (if all the living mice developed mammary cancer, the incidence would be increased to 60 per cent). Six of ten females which were bred and permitted to nurse their young died with mammary cancer.

Thus, no critical comparative data have been published which would suggest that force breeding, rapid breeding with the prevention of nursing, has any precipitating effect upon the genesis of mammary cancer in mice. It has been suggested (20) that force breeding may produce duct stasis in the mammary system and with the retention of milk, an irritating chemical effect may be produced upon the mammary gland epithelium. From our experience in obtaining lactating mammary tissue for studies on

the milk agent, there is little secretion of milk during the first day and the amount of stagnation would probably be less than in females which had nursed for a longer period, perhaps even until the young are weaned.

If the difference between the incidences of tumors seen in virgin and breeding females of the same stock was due primarily to stagnation, one would expect that castrated males with transplanted ovaries would develop few, if any, mammary tumors. In one study the incidence in the latter group approximated the incidence observed in breeding females of the same stock and the average cancer age was only one month later (91, 54).

Carcinogenic hydrocarbons have been tested as to their ability to induce mamary tumors in mice and it has been observed that these tumors may be produced in animals of some strains which are known to have low spontaneous incidences (148, 149, 98, 147, 99) as well as in stocks with high incidences (112, 59, 68–69, 96–97, 113, 117–118). Such tumors may also be produced in mice of some susceptible strains which do not have the milk agent although the incidence has been found to be higher when the females possess the agent (99).

Squamous metaplasia has been reported in carcinogen induced mammary cancer by some workers (113, 149, 98, 131, 99) and the histogenesis of these induced tumors may be the same regardless of whether or not the mice have the milk agent (99). Further studies must be completed to determine if the two types of tumors may be the same since the susceptibility to spontaneous and induced mammary tumors cannot be correlated and "the histological evidence does not favor the concept that the carcinogen accelerates the sequence of alterations seen in the histogenesis of spontaneous milk agent tumors in mice" (99).

Various references have been made to the inherited hormonal influence, another inherited factor which operates in the development of mammary cancer in virgin mice and is mediated through the genic control of the hormonal stimuli (55, 86, 54). That it probably is not determined by the same genes as the inherited susceptibility for mammary cancer has been indicated (54).

In addition to the association between the adrenal cortical changes in ovariectomized mice of some stocks and the inherited hormonal influence (143–145), certain other possible physiological effects have been noted. Deringer, Heston, and Andervont (63) compared the virgin females of two inbred strains and found a correlation between the age at which the vagina opened and the incidence of mammary cancer.

By observing spayed F_1 hybrid females produced by reciprocal matings between the A (incidence as virgins, 4 per cent) and the Z or C_3H (63 per cent as virgins) stocks and transplanting ovaries from the parental strains and F_1 hybrid females, it has been possible to study the effect of ovarian secretion. In the F_1 females the grafted ovaries would be subjected to the same pituitary stimulation and the hormones that were produced would be metabolized in the same manner and stimulate the same substrate. During the first ten months of the experiment there was no difference in the vaginal smear data or the degree of variability of the estrus cycles in the three groups.

At the time the preliminary report was given the incidence of mammary cancer in the ovariectomized F_1 females with ovaries from donors of the A stock was less than half that seen in the F_1 hybrids bearing Z or hybrid ovaries. At present, although no significant difference is apparent in the three groups, the mice with ovaries from the A strain developed their mammary tumors at a much later age than did the mice of the other groups. Thus, it seems probable that the physiological effects of the inherited hormonal influence are partially, but by no means completely, expressed through the ovarian secretion (90 and unpublished data).

The incidence of mammary cancer may not remain constant in an inbred stock of mice over an extended period and it is unusual to find that mice of the same strain will show the same incidence when they are maintained in different laboratories (for literature see 49). Such data emphasize the importance of having controls to compare with each group of experimental animals. The results obtained by another in a different laboratory should never be referred to as control data.

The effect of diet on the development of mammary cancer in mice has received considerable attention during recent years. If mice of the same cancerous strain are given different diets, each considered to be adequate, different incidences may be obtained which are of statistical significance (27). The animals on the diet which gave the higher incidence had larger litters, the mortality among the young was less, and the females were younger when they gave birth to their first litters. The higher incidence was attributed to the better physical condition of the animals as perhaps influenced by diet.

The influence of underfeeding (138, 150), caloric restriction (157, 151–154, 167), low cystine (164, 168, 165, 163) and low lysine (166) diets have been studied.

Because few of the animals on the restricted diet became pregnant (157) and the histological examination (88) of the sexual organs showed the picture of pseudo-hypophysectomy, it has been concluded that the mechanism by which caloric restriction *per se* inhibits the development of mammary cancer is probably pituitary insufficiency producing (a) a lowered level of ovarian secretion and (b) a relative refractoriness of the mammary gland to estrogenic substances. This conclusion would be in agreement with the findings of others (168, 165, 167, 163, 116). In one of these experiments none of the mice on a low cystine diet developed mammary cancer but pellets of diethylstilbestrol induced tumors in 45 per cent of the mice on the same diet (165). Further, it has as yet been impossible to effectively control the development of mammary cancer by diet without also effecting an almost complete sterility.

Other factors may exert an influence upon the genesis of these tumors. Fuller, Brown and Mills (74) and Wallace, Wallace, and Mills (160, 161) considered the effects of temperature and determined that animals maintained at 90°–91° F. gave rise to fewer tumors than did the mice kept at either 65° F. or 70°–75° F. In view of the results secured in the underfeeding and restriction experiments, the fact that the mice which were housed at high temperatures consumed only about half as much food as

did the others and showed growth suppression, may be of importance in the interpretation of their results.

Andervont (9) noted that segregated mice showed a higher incidence and an earlier average cancer age than did those kept eight per pen. Differences in the frequency and duration of the estrus cycles were seen and it was assumed that the results might be explained upon environmental factors which exerted an influence upon the hormonal stimulation.

The mammary tumor milk agent was found by fostering the new-born young of females of cancerous strains (29). Low incidences were usually obtained in the progeny and descendants of the fostered mice but if they were nursed by females of cancerous stock, approximately the same incidence was noted in these mice as in the original cancerous stock. If test animals (susceptible mice without the milk agent) are either fed or injected with extracts of tissues from mice with the agent, an increased incidence is obtained.

Based upon the development of mammary cancer in the test animals, the agent may be recovered from every tissue which has been examined to date. These include the spleen (30, 34, 16, 64), thymus (30, 34), lactating mammary gland (37, 14, 22), Harderian gland (57), whole blood (172), and liver (52). Although some workers (78) have had little success following the use of either whole blood or blood fractions, we have been able to obtain a considerable incidence by injecting either a suspension of blood cells or serum (48). The agent can also be adsorbed onto red blood cells from mice lacking the agent which have been added to the serum from cancerous animals (51, 52). It can usually be recovered from spontaneous mammary cancer (41, 23, 14) and from these tumors after they have been transplanted for ten passages in mice which did not have the agent (53).

The agent has been found to be active following lyophilization (41), filtration (43, 44, 14), treatment with glycerin (43, 14), and desiccation (49, 65–68). Following the injection of cell free extracts of cancerous tissue into the developing chick embryo,

activity has been seen in both filtered and unfiltered yolk after one passage and in unfiltered yolk after nine serial passages in eggs (53 and unpublished). The agent loses its activity when maintained at temperatures of 56° C. or above for thirty minutes or longer (14, 23).

Evidently even susceptible animals may become resistant to the agent with increasing age (7, 16, 44, 12) although it has been reported that it is possible to overcome this resistance by giving repeated injections of the agent (66).

From the results obtained in the earlier studies it was considered that the incidence and average cancer age in the test animals were dependent upon the amount of the agent which the animals received. More recently (48), however, it has been determined from serial dilution studies that the mice of comparable ages may show a higher incidence when they received 10^{-4} gram equivalent than when given either 0.1 or 0.2 gram equivalent; in younger mice no significant difference may be noted. An extract diluted 10^6 will contain a sufficient amount of the agent to result in the development of mammary cancer (50–52) and in one experiment an incidence of 35 per cent was found (unpublished data).

In another experiment the extracts of lactating mammary tissue showed greater activity than did those of either spontaneous cancer or transplanted mammary cancer (22). When test animals of another genetic constitution were injected, no significant variation was noted between those which received extracts of mammary gland or spontaneous mammary cancer but both groups had higher incidences than were seen in the mice tested with either suspended blood cells or serum from cancerous mice of the same stock (51–52).

Several centrifugation studies have been reported (158, 60, 95, 22) and in one it was found that essentially all of the agent was sedimented at 18,000 g. for a period of one hour (22). The greatest activity was found in the fractions containing the larger particles or the microsomes at dilutions of 10^{-2} to 10^{-6} gram equivalents (22).

Thus, in experiments to determine the activity and/or the concentration of the milk agent either in mice of different cancerous strains or in diverse tissues from the same animal, the results may be influenced by the age of the test animals at the time of injection, their genetic constitution, the concentration of the extract, and the fraction of the tissue which may be used. It is probable that environmental factors are important.

Antibodies against the agent may be stimulated in rabbits and rats by the injection of extracts of either spontaneous or transplanted mammary cancer (14, 79–80) and the serum from these immunized animals will neutralize the agent *in vitro*. Mammary cancer did not result in the mice which received the serum previous to the injection of the agent (14) but if the animals had obtained the agent by nursing, the injection of the immune serum at a later date has failed to prevent the development of mammary cancer. The serum has a cytotoxic effect upon a suspension of mammary cancer cells *in vitro* (80).

As yet it has been impossible to find morphological evidence of the presence of the milk agent in the mammary glands prior to the development of the precancerous lesions. It has been claimed that the formation of lateral buds along the ducts of the mammary glands was influenced by the presence of the agent (156) but these observations could not be corroborated in a study of the architecture of the glands from fostered and unfostered females of other cancerous stock (89). These conflicting results may be due to the use of mice from different stocks which do not have the same arrangement of the duct system (77). The use of estrogenic hormones likewise has failed to reveal consistent variations in the structure of the mammary glands which might be ascribed to the action of the milk agent (137).

There are no experimental data which would establish that any other type of cancer in mice may be controlled by nursing.

Should these observations made on mice be applicable to breast cancer in humans, for which we have no evidence at this time, it would be necessary to bottle-feed only one generation to eliminate any milk-transmitted agent. If a human milk agent should exist

and it had the same characteristics as the agent in mice, it could also be obtained by blood transfusions.

The human data on breast cancer would not indicate that the prevention of breast feeding would tend to increase the incidence of breast cancer in the mothers. Waller (162) followed a group of primiparous women and found that 28 per cent had stopped breast feeding by the end of one month and 42 per cent before the third month had passed. Whereas in mice parous females usually have a higher incidence of mammary cancer than do the virgin females of the same stock, human data show that unmarried women are slightly more likely to develop breast cancer than are married women although there may be no difference in the incidence of gastro-intestinal cancer in the two groups (84). They found, as have others, that a late menopause was more frequent in women with breast cancer than in those who did not develop the condition.

Again if we might make use of the data from animals, a possible explanation might be suggested for the higher incidence in unmarried women on the basis of the inherited hormonal influence. It probably would have to follow that a considerable proportion of these individuals would not marry, perhaps for some physiological or psychological cause of hormonal origin. Clinical improvement in some males with prostatic cancer following castration may be due to an analogous inherited hormonal complex in humans.

Human surveys are being made to determine any possible association between nursing and the genesis of breast cancer in humans but it will require many years to obtain this material.

SUMMARY

Several inciting factors have been demonstrated to be responsible for the development of mammary cancer in mice. The hormones control the growth and development of the mammary glands without which mammary cancer will not result. The exact rôle of the inherited susceptibility has not been determined but it may have something to do with the propagation and trans-

mission of the mammary tumor milk agent, determine the sensitivity of the mammary tissue to the action of the hormones and/or the milk agent, etc.   Another inherited factor, namely the inherited hormonal influence (genic control of the hormonal stimuli) acts in producing a high incidence in virgin females in association with the other factors.   Since the milk agent, normally obtained by nursing, is filterable and sedimentable, can propagate in the living cell, and is an antigenic entity, it seems logical to classify it with the filterable viruses.

By studying various strains of mice with and without the milk agent under different experimental conditions, it has been shown that all of the inciting factors are required before a high incidence can be obtained.   This being the case, we are not justified, from our present knowledge, to term any one the "inciter" for mammary cancer in mice since in the absence of any one of the causative factors, a low incidence will be obtained.   The most effective method to prevent the development of mammary cancer in mice is by elimination of the agent in the milk by controlled nursing.

The incidence of mammary cancer in mice may be influenced by under-feeding, certain specific dietary restrictions, and other environmental factors.   The physiological effect is probably through a suppression of the normal hormonal stimulation. While this type of cancer may be practically prevented in mice on some restricted diets, it has not been shown that this can be accomplished without altering the fertility of the animals as well.

Whether or not these observations on mice may serve as a basis for the control of breast cancer in humans must be determined by further study which may take years to complete.

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PHYSIOLOGICAL INFORMATION GAINED FROM STUDIES ON THE LIFE RAFT RATION[1]

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IN THIS lecture I will present briefly and somewhat at random items of physiological interest gained from studies on the life raft ration which were carried out at the Massachusetts General Hospital. A part of the laboratory work was performed at the Children's Hospital. Dr. Allan Butler was, in the stern language of CMR, the responsible investigator. My position as a guest member of his team was very pleasantly irresponsible.[2] These studies were undertaken before the merciful invention of methods of obtaining water from sea water. Their premise was therefore a limited weight allowance per man per day for food and water. Since survival for fasting is several times longer than for thirsting, the physiological benefits of food, under the circumstance of a restricted overall allowance for both food and water, must be appraised in terms of cost to the water balance of the body. The obviously indicated first step in this study was to define the minimal water requirement in the state of fasting. The second step was to observe, in subsequent experiments, alteration of this requirement produced by intake of a given kind of food. The data thus gained produced definition of body water

[1] Lecture delivered March 20, 1947.

[2] The members of the group were: Butler, A. M., Gamble, J. L., Talbot, N. B., MacLachlan, E. A., Appleton, J., Fahey, K., and Linton, M. A., Jr.

A complete account of these studies has not yet been published. Some of the findings have been briefly reported in several papers with the following titles: The Water Requirements of Castaways, Gamble, J. L., Proc. Amer. Philosophical Soc., 88: 151, 1944. Measurement of the Renal Water Requirement, Gamble, J. L., and Butler, A. M., Trans. Assn. Amer. Phys., 58: 157, 1944. Services of Dextrose in Parenteral Fluid Therapy, Butler, A. M., Gamble, J. L., Talbot, N. B., MacLachlan, E. A., and Appleton, J., Trans. Amer. Ped. Soc., Amer. J. Dis. Child., 72: 443, 1946.

deficit resulting from replacement of part of a minimal, or sub-minimal, water allowance by food.

We were provided with healthy young men as subjects who were courageously willing to undergo periods of thirsting and fasting, and were thus given an unusual opportunity to obtain quantitative description of the components of the water exchange in these states.

THE WATER EXCHANGE OF THIRSTING AND OF FASTING

The diagrams in figure 1 are constructed from data obtained over a 4-day period of thirsting, a 6-day fast with ample water intake and a 6-day period during which a moderate intake of carbohydrate was provided along with water. The values recorded in the diagrams are cubic centimeters of water per 70 kilograms of body weight per day.[3] The diagrams are built on the basis of a daily insensible water loss of 1000 cc. This large component of water outgo was held stationary because of its independence of the differing conditions of the several experiments, the relation of which to the other components of the water exchange the diagrams are intended to describe.[4] The removal of body water, BW, is defined by the values found for the other components of the water exchange. As indicated by the broken line, this water is taken from both the extracellular and the intracellular body fluid compartments. Estimation of the loss of extracellular water is derived from measurement of the loss of chloride from the body. The remainder of BW is taken as intracellular water.

As shown by the diagram for the thirsting period, obligatory expenditure of water composed of insensible water and urine water is about 1500 cc. A small part of this outgo is covered by water produced by oxidation of body fat and protein. The remainder is composed of preformed body water which was

[3] The average values per day are for the last three days of the thirsting period and for the last four days of the fasting and carbohydrate periods.

[4] This involved adjustment of the found value for BW in the thirsting period and for UW in the fasting and carbohydrate periods to the small departures of the found values for IW from 1000 cc.

withdrawn to the extent of about 1300 cc. daily. It may be mentioned here that, according to experiments with animals, a loss of 38–40% of body water marks the survival limit for dehydra-

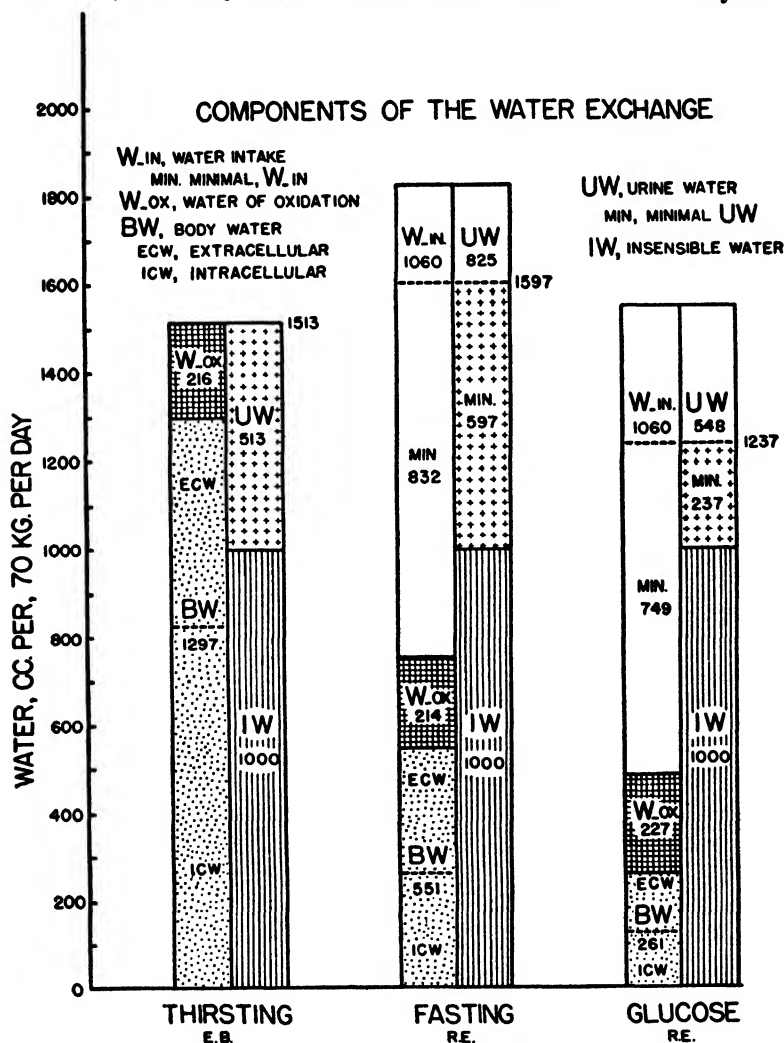COMPONENTS OF THE WATER EXCHANGE

FIG. 1.

tion by thirsting.   For a 70 Kg. man this maximal loss would be about 19 liters and, at the rate of a daily loss of 1300 cc., survival expectancy would be 14 days.

The next diagram describes the water exchange as found for the state of fasting when the additional circumstance of thirsting has been removed.   Although withdrawal of body water is extensively reduced, a considerable removal remains.   This is explained by the loss of body fluid solutes which is incidental to fasting.   Loss of body protein by oxidation is found to be accompanied by removal of cell water to the extent required to preserve the normal concentration of protein in cell fluid.   Measurement of this physiological loss of intracellular water can be derived from nitrogen outgo in the urine.   There is also removal of extracellular water which closely parallels a loss of the extracellular fluid solutes.   So that there is within the body preformed water available for expenditure to this extent and also water produced by oxidation of body fat and protein.   What remains to be defined is the quantity of water which must be provided in order that body water will not be withdrawn beyond the physiologically permissible extent.   The castaway must use water as economically as possible which means that expenditure must be reduced to the physiological minimal.   Under ordinary environmental circumstances and when there is very little physical activity the insensible expenditure of water has an approximately stationary value which we have taken as 1000 cc.   With a usual water intake, under guidance of the sensation of thirst, the water removed by the kidney in order to preserve balance of the water exchange is in large surplus over the quantity of water required to place the daily load of solutes in the urine.   So at this point we are asked to define the minimal renal requirement for water.   This will require definition of the daily solute load and of maximal concentration of solutes in urine.   This information can be reached from a measurement of the freezing point depression in the urine.   The steps in estimating the renal water requirement are illustrated in table 1.

The physical chemists tell us that if we divide degrees of freez-

ing point depression by this factor, 1.86, we obtain osmolar concentration. If we then multiply osmoles per liter by milliliters of urine per 24 hrs. we define the total output of solutes as milliosmols. That is, we have an overall statement of the kidney's excretory assignment in terms of the physical unit which correctly measures this physiological performance. If we then divide this load by a value taken as maximal for the ability of the kidney to concentrate solutes in the urine we find the minimal volume required to remove it. We have taken 1.4 osmolar as the physiological maximal because it was the average value found for our subjects under the requirement for conservation of water

TABLE 1

Estimation of Minimal Renal Requirement for Water

Example
Freezing point of urine −1.43°
Urine Volume 990 ml.   Solids 41 gm.

$$\frac{1.43}{1.86} = 0.77 \text{ osmols per liter}$$

$0.77 \times 990 = 762$ milliosmols Total Solutes

$$\frac{7.62}{1.4} = 544 \text{ ml. Minimal Urine Volume}$$

$(544 \times 1.035) - 41 = 522$ ml. Min. Urine Water

produced by periods of water deprivation. The remaining step is to subtract the weight of urine solids from the weight of minimal volume and so reach a measurement of minimal urine water. The specific gravity value used here was found to correspond approximately to an osmolar concentration of 1.4 in urine from our subjects.

The value found for minimal urine water for the subject R.E. over the period of fasting (figure 1) together with IW defines an obligatory expenditure of about 1600 cc. By carrying the broken line across the diagram we obtain the measurement we are after, minimal water intake. We found approximately this value for our other four subjects. So that according to this method of estimation, the minimal water requirement for the

state of fasting under ordinary environmental conditions and approximately basal energy expenditure may be taken as about 800 cc. per day. It may be noted that, owing to water available within the body, intake is needed to the extent of only about one-half of obligatory expenditure and so is a much smaller quantity than the state of nutritional equilibrium requires.

<div align="center">CARBOHYDRATE AND THE WATER EXCHANGE</div>

The third diagram in figure 1 describes the alterations of the components of the water exchange when a moderate intake of carbohydrate is provided. The subject was given 100 gm. glucose daily, along with the same water intake as in the fasting period. We find a large reduction of the renal water requirement and, on the other side of the diagram, reduction of body water removal to about one-half of the quantity found for fasting. The lowering of minimal urine water is of larger extent than the reduction of body water available for expenditure with the result that minimal water intake is less than was found for fasting.

Explanation of this gainful transaction is found in effects of glucose on the quantity of solutes claiming removal in the urine. When glucose is given the losses of the normal body fluid solutes incidental to the state of fasting are reduced by about one-half with corresponding reduction of minimal urine water and of available body water. The absence of the abnormal solutes which derive from the ketosis of fasting produces further reduction of the renal water requirement but does not alter expendable body water as determined by the extent of loss of the normal body fluid solutes. So we find that it is the antiketogenic effect of glucose which produces the larger lowering of minimal urine water than of body water presenting for removal. According to the values given on the chart, the resulting reduction of the water intake requirement is 83 cc. which is almost as large as the quantity of glucose given. In other words, we find that we can substitute 100 gm. of glucose for 100 cc. of the minimal water intake found for fasting at almost no cost to the water exchange. Since

100 gm. of glucose completely prevents ketosis, this manipulation of the water exchange cannot be carried further.   Additional replacement of minimal water intake by glucose will cause equivalent water deficit.   The several benefits from 100 gm. of carbohydrate are, however, of great importance to the castaway.   Besides prevention of ketosis, there is a large saving of the body fluids which defines corresponding conservation of protein and of the other components of the body fluids, with resulting extension of survival beyond the expectancy for fasting.   There is also a large contribution to morale.   The cheerfulness and sense of physical effectiveness of our subjects when they were given glucose was in striking contrast with the unhappy lassitude of fasting.

These data quite clearly establish the eligibility of a moderate quantity of carbohydrate for place in the life raft ration.   Since survival for fasting rests mainly on the fat stores of the body and is much longer than for thirsting, we have an á priori verdict against the provision of fat at a cost to the minimal water requirement.   Evidence of the physiological futility of protein in a sub-energy maintenance ration will be presented further along in this lecture.   So our conclusion was that a survival ration, the weight limit for which must be 800 gm. per day or less, should contain 100 gm. carbohydrate and the remainder of the weight allowance should be devoted to water.

EXTRA-RENAL WATER LOSS

Our derivation of the water intake requirement from values found for water available within the body and for minimal outgo in the urine rests on a stationary loss of water by way of the lungs and skin.   It is obviously important for the castaway that this larger component of water expenditure be held at somewhere near its basal value.   Extension of IW will cause a corresponding increase of the water requirement.   Men adrift on a raft in hot regions are exposed to the hazard of a greatly increased vaporization of water from the surface of the body in defense of body temperature.   In this situation there is therefore urgent

need for the employment of measures which will promote removal of heat from the body by other processes, and thereby prevent or limit water wastage which can be of disastrous extent.
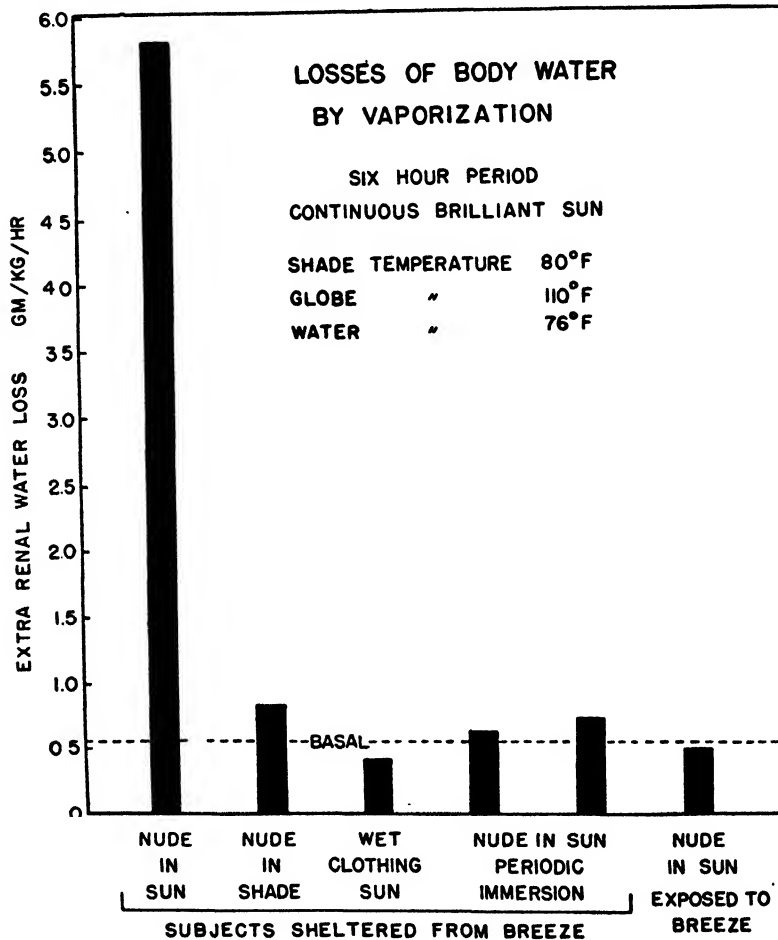

FIG. 2.

Some of the results of a field study which was undertaken with the purpose of demonstrating how large the expenditure of water

by excessive vaporization may be and the importance and also
the effectiveness of several simple ways of keeping castaways cool
are shown by figure 2.   We have here five men on a raft moored
in a small cove at the upper end of Cotuit Bay on Cape Cod.   It
was a moderately hot summer day.   The raft was provided on all
sides with a screen of stout cloth which almost entirely excluded
breeze.   Extra renal water loss is recorded as grams of water
per kilogram of body weight per hour.   The values found for the
individual subjects are shown by the black columns.   These val-
ues were obtained by the simple procedure of weighing the sub-
ject unclothed at the beginning and at the end of 5- to 8-hour
periods and measuring, in terms of weight, water and food in-
take and the outgo of urine.   The subjects drank water accord-
ing to thirst and were given a noontime meal of 50 gm. of butter-
scotch.   The broken line across the chart defines the average of
the values for insensible water expenditure found for the five
subjects over an 8-hour period during which they reclined ashore
in the shade of pine trees.   This was taken as the basal or irre-
ducible expenditure.   We were quite proud to have come so
close, in our outdoor laboratory, to the standard value, 0.5 gm.,
established for basal insensible water loss by the careful work of
DuBois.   The subject whose expenditure is measured by the tall
column did nothing to avoid wastage of body water.   He lay be-
hind the wind screen exposed to the sun.   He sweated copiously
and lost water at ten times the basal rate.   The water loss for
the 6-hr. period was 2400 cc.   So that he squandered in six hours
three times the daily intake requirement for basal expenditure.
Loss of water by a thirsting castaway at this rate over 12-hr.
periods on successive days would bring death by dehydration
within about four days.   The next subject lay in the shade of
a garden umbrella.   Reduction of environmental warmth from
110°, as recorded by the so-called globe thermometer, to 80° did
not quite prevent water loss above the basal rate.   Promotion of
heat removal by conduction, gained by periodic immersion in the
sea, held the water loss at approximately the basal mark.   The
simple device of wetting the subjects clothing with sea water was

found to provide the most dependable defense of the basal rate. This amounts to substitution of vaporization of sea water for the vaporization of body water. The first subject on another and similar day sat in a rowboat clad in bathing trunks and was expose to a light breeze which crept into the cove. The two measurements from this subject show you very strikingly the large effectiveness of breeze in promoting heat removal by convection. Fortunately for the castaway there is usually breeze. His greatest hazard is hot windless weather. Then disaster will come very quickly unless measures are taken to prevent excessive vaporization of body water.

The data from these very simple experiments remind us of the wide extensibility of water expenditure by vaporization when there is departure from the environmental conditions for preservation of its basal value. This is not without import in clinical medicine.

SEA WATER EXPERIMENTS

The plight of a thirsting castaway raises the ancient question; can survival be extended by drinking sea water? According to legend this question has been decided in the negative. We were, nevertheless, commanded to examine the physiological premises for the poetical postulation: "Water, water everywhere, nor any drop to drink." The initial premise is a quite simple one. If the kidney can remove the sea water solutes at an osmolar concentration above that of sea water, the body will gain water which can be applied to extra-renal expenditure. The sea water used in these experiments came to us from the Oceanographic Institute at Woods Hole. So it is perhaps not surprising that we found it to be almost precisely an osmolar solution.

The theoretical water gains from ingestion of a liter of this sea water according to solute concentration in the urine are shown in table 2. If urine is secreted at the average maximal, 1.4 osmolar, the quantity of water required to remove the solutes from a liter of sea water is 714 cc. and so there will be 286 cc. left over. A liter of sea water is, however, beyond the limit of gastro-intestinal tolerance. We found that the daily quantity

TABLE 2

Renal Water Requirement for Removal of Solutes of 1.0 Liter of Ingested Sea Water

(Solutes in sea water 1.0 os M/L)

Urine conc.	Water Required	Water Gained
os M/L	*cc.*	*cc.*
1.0	1000	0
1.1	910	90
1.2	834	166
1.3	770	230
1.4	714	286

of sea water which our subjects could drink without gastro-intestinal qualm was 500 to 600 cc. The water gain from an intake of 500 cc. of sea water should be about 150 cc. We undertook to verify this postulated gain by comparing the volumes of urine found for a subject while thirsting and in a subsequent period during which he drank 500 cc. of sea water daily. Attainment of the theoretical gain will require the secretion of urine at maximal solute concentration in both experiments. Also for the water gain to be physiologically valid the sea water solutes must be completely removed.

According to the data in table 3, the performance of the subject was on the mark. Urine solute concentration was maximal

TABLE 3

Sea Water Experiment

Intake	Urine solutes		Urine water
	Osmols per liter	Total milliomols	
			ml.
Thirsting	1.42	758	507
Sea Water, 500 cc.	1.46	1275	822
		517	315
		Water Gain, 500−315 = 185	

in both experiments.　Since the sea water was an osmolar solution, 500 cc. contained 500 milliosmols of solutes.　The addition to the outgo of solutes in the urine was 517 m-osmol.　Apparently
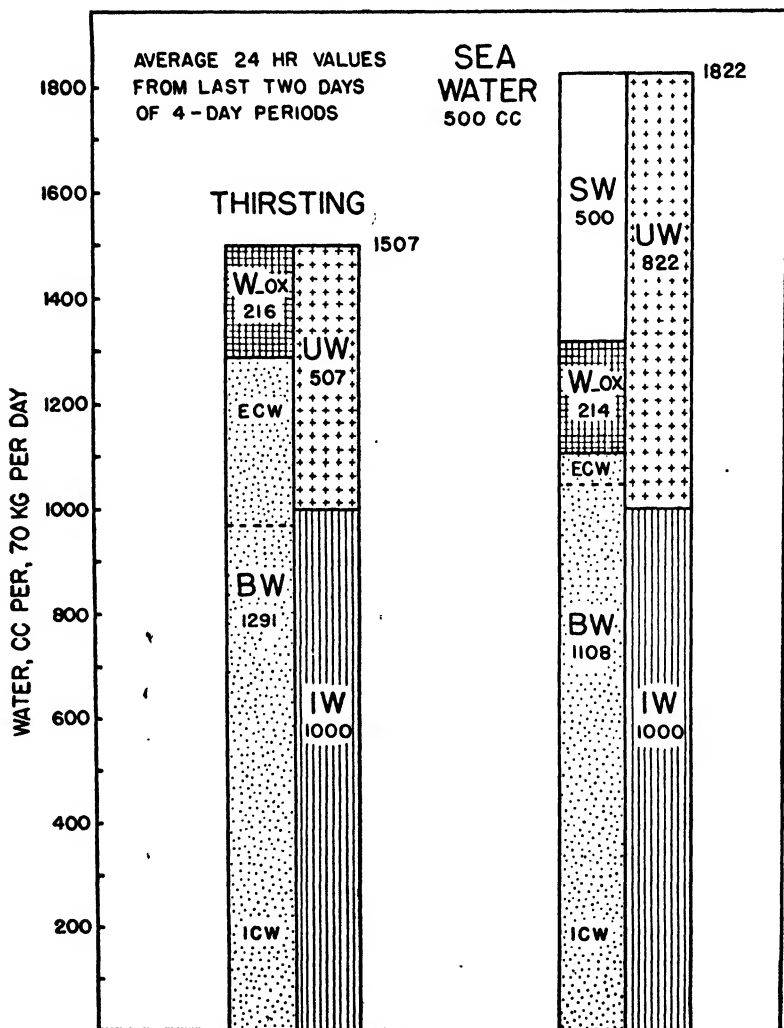
FIG. 3.

the kidney removed the ingested solutes with an admirable accuracy. Urine water for the thirsting period was 507 cc. On this basis, if there were no water gain from an intake of 500 cc. of sea water, urine water should be increased to 1007 cc. The volume found was 822 cc. Subtracting the increase in urine water from intake defines a water gain of 185 cc.

The water gained from sea water by the kidney is applied to reduction of the rate of body water loss found for thirsting. The extent of this reduction is shown by the diagrams in figure 3. It may be estimated that this small saving of body water would prolong survival by two or three days beyond the two weeks predicted for thirsting.

The small but appreciable water gain from sea water which these experiments demonstrate would seem to command a thirsting castaway to drink the pint of sea water which gastro-intestinal tolerance permits. When, however, we examine partition of body water loss we find effects of a sea water intake which give us pause. As has been mentioned, the estimations of loss of extracellular water shown in the diagrams above the broken line are derived from measurement of chloride loss from the body. The remainder of body water loss is taken as intracellular water. As might be expected from the large intake of the extracellular ions, sodium and chloride, which sea water provides, the loss of extracellular water, as defined by chloride deficit, is found to be extensively reduced. As can be seen in the diagrams this reduction is larger than the overall reduction of total body water loss, with a result that withdrawal of intracellular water is increased, and, as further evidence of this, we find an increased outgo of potassium in the urine.

What is going on as regards the balances for the individual electrolytes is shown by figure 4. The stippled columns measure the daily losses during thirsting and the striped columns the deficits found in the sea water experiment. The large intake of sodium and chloride which sea water provides extensively reduces the rate of loss from the body of these extracellular ions. The sea water content of potassium is relatively very small and we

find an increased deficit of this prominent component of intra-cellular fluid. This loss is, however, approximately offset by an actually positive balance for another, mainly intracellular ion, magnesium, of which sea water provides a considerable intake.

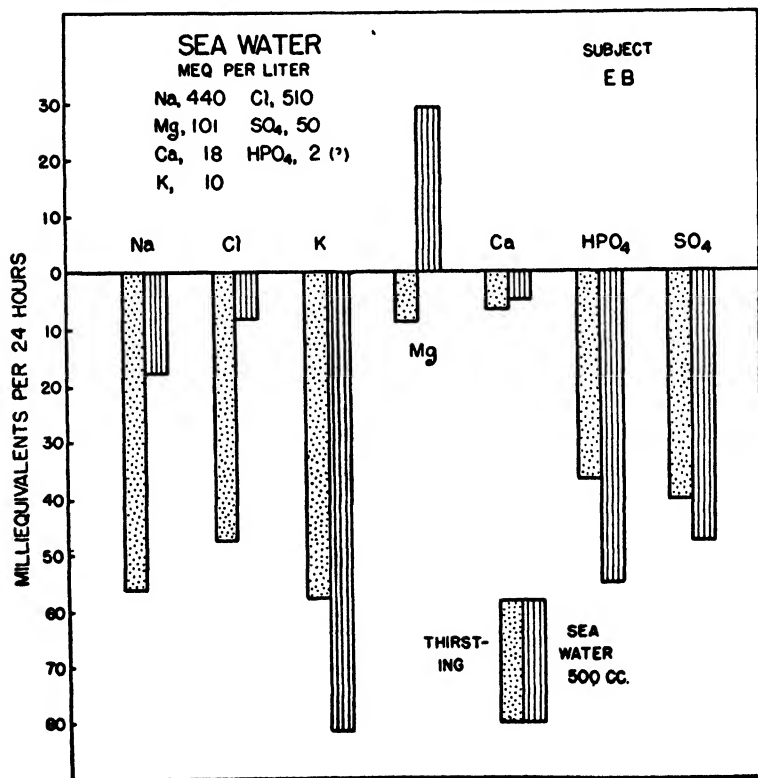EFFECTS OF SEA WATER ON BALANCES ON INORGANIC IONS

SEA WATER
MEQ PER LITER

Na, 440   Cl, 510
Mg, 101   SO₄, 50
Ca, 18   HPO₄, 2 (?)
K, 10

SUBJECT
E B

FIG. 4.

Calcium balance is not appreciably altered. There is some ex-tension of deficits of the predominantly intracellular anions phosphate and sulfate. So we find that, although the addition to the total output of solutes in the urine when sea water is drunk

is closely equivalent to intake (table 3), the composition of this addition is not quite the same as that of sea water owing to this swapping of electrolytes between sea water and the body fluids. The conspicuous event is the inverse alteration of the sodium and potassium balances.   Similar behaviour of these two ions has been observed under various conditions.   So that the increased potassium deficit might be primarily related to the change in sodium balance or it might be the result of direct displacement of potassium from cell fluid by retention of magnesium.   At any rate these data make it clear that ingestion of sea water sets up a process of alteration of the composition of intracellular fluid, and of reduction of volume relative to the volume of extracellular fluid.   Whether or not his process, which will proceed gradually, is of such physiological disadvantage as to offset a small but valuable overall water gain from sea water we are quite unable to say.   Judging the evidence conservatively the verdict of legend against the ingestion of sea water by castaways would seem to be sustained.

THE PHYSIOLOGICAL REQUIREMENT IN PARENTERAL FLUID THERAPY

Much of the information from these experiments is relevant to the clinical problem of sustaining the body fluids when an oral intake of water and substances cannot be provided.   In parenteral fluid therapy glucose solution is used to cover the current obligatory expenditures of water by the body.   As shown by the data which have been presented, glucose on its own account, performs several physiological services which together contribute to conservation of the body fluids.   It is therefore desirable to know how much glucose should be provided, along with water, in order to gain these benefits to their maximal extent.   Our studies on the life raft ration produced definition of this requirement.

Development of ketosis over 6-day periods of fasting is shown in figure 5 by the progressive increase of the total organic acid outgo in urine above the values found on the first day.   Ketone acids do not begin to appear in the urine until the second day of fasting.   The other data on the chart are from 6-day periods

ORGANIC ACIDS
IN URINE
EFFECT OF
CARBOHYDRATE
INTAKE

FASTING

FASTING

GLUCOSE
GM
1, 50
2, 100
3, 100
4, 200

R.E. ———●———
M.G. ●---●---●

MEQ.
24 HRS.

DAY   1   2   3   4   5   6
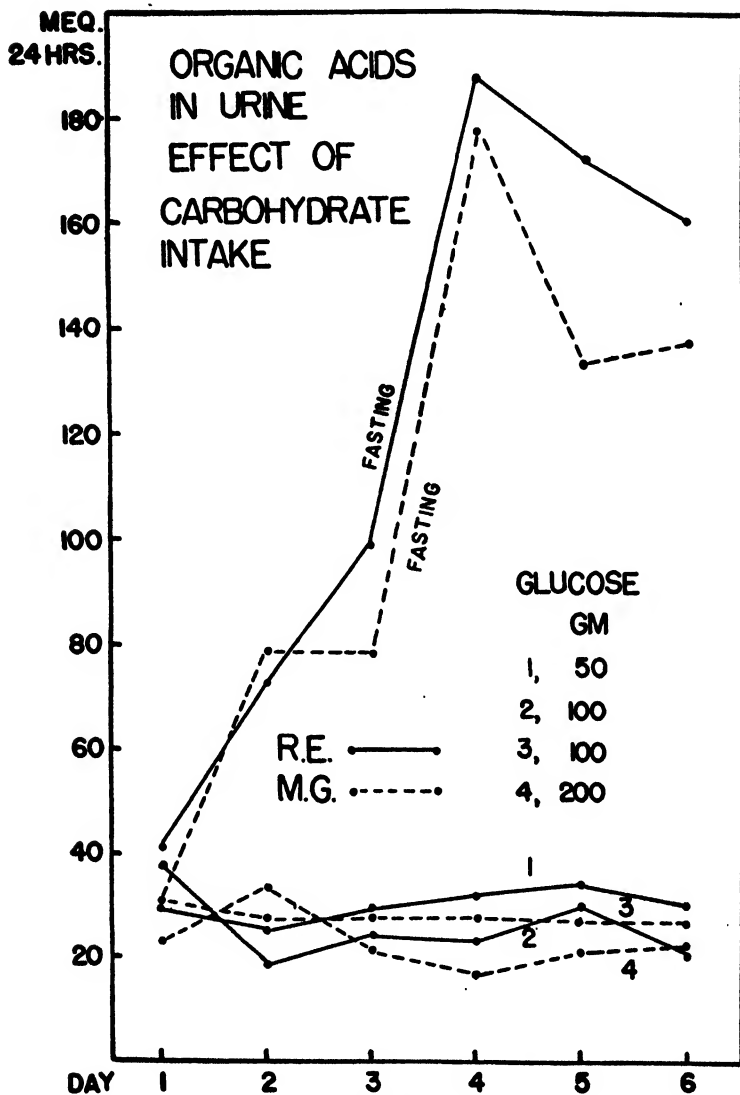
Fig. 5.

during which glucose was provided at several levels of intake. According to these experiments, an intake of 100 gm. daily will dependably prevent the ketosis of fasting; 50 gm. is almost as effective.

The data in the upper section of figure 6 describe the well known protein sparing effect of glucose. The lowest line measures the progress of consumption of body protein across a 6-day period of fasting. This amounts to something more than 400 gm. with an accompanying removal of about 1200 cc. of intracellular water. An intake of 50 gm. of glucose daily causes a considerable reduction of the protein loss. When 100 gm. is provided the loss is reduced to about one-half the extent found for fasting. When intake is increased to 200 gm. there is very little further protection of body protein. So that, according to these data, the maximal protein sparing effect of glucose is approximately gained by providing a daily intake of 100 gm. and amounts to prevention of about one-half the oxidation of body protein which is incidental to the fasting state. This limit to protein sparing is understandable. The daily loss during fasting is about 80 gm. per 70 Kg. Maximal sparing by glucose brings the loss down to approximately 40 gm. which is just about the minimal protein intake required for nitrogen balance under normal nutritional circumstances. In other words, this is just another way of defining the minimal level of protein metabolism.

The data in the lower section of this chart describe an unexpected effect of glucose; an extensive conservation of the extracellular electrolyte, sodium. The loss of sodium by this subject over a 6-day fast was about 350 meq. and defines a removal of 2½ liters of extracellular fluid. An intake of glucose reduces this loss by something more than one-half and maximal sparing is approximately gained by providing 50 gm. of glucose daily. We have no explanation to offer for this effect of glucose. According to the evidence presented by the next chart (figure 7), it is not related to the anti-ketogenic effect of glucose. As shown by these measurements the addition to the organic acid excretion during fasting composed of the ketone acids is covered with a quite
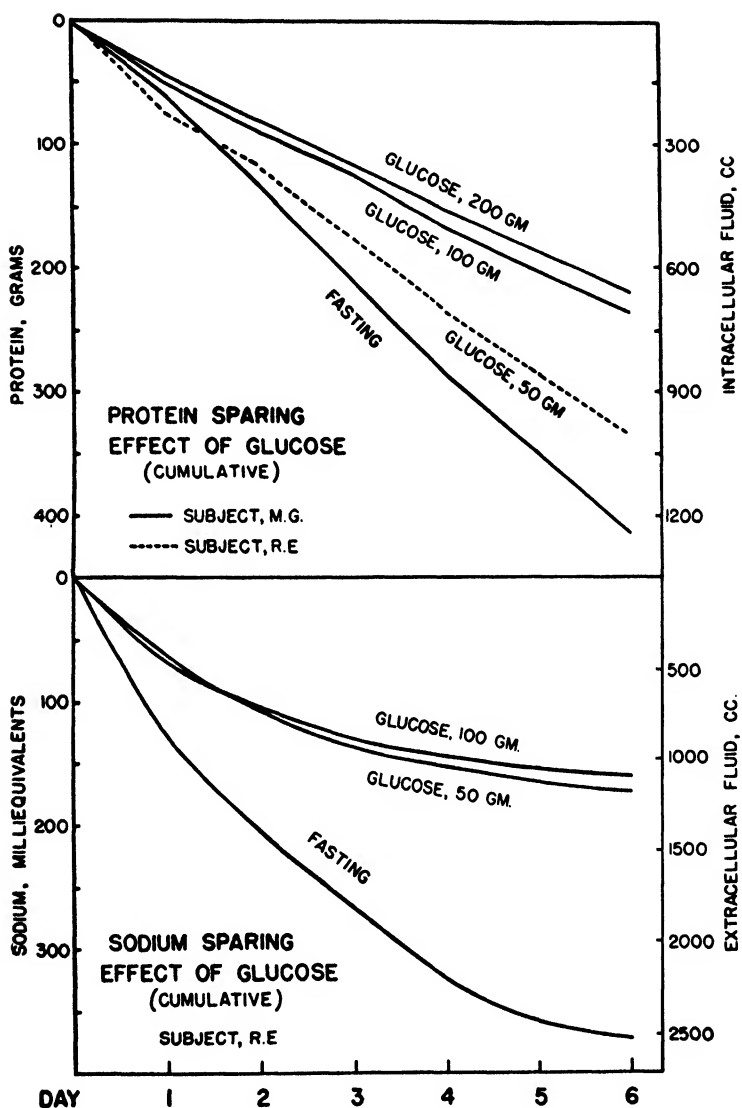
FIG. 6.

beautiful accuracy by extension of ammonium production to-
gether with an increased titratable acidity of the urine.   The
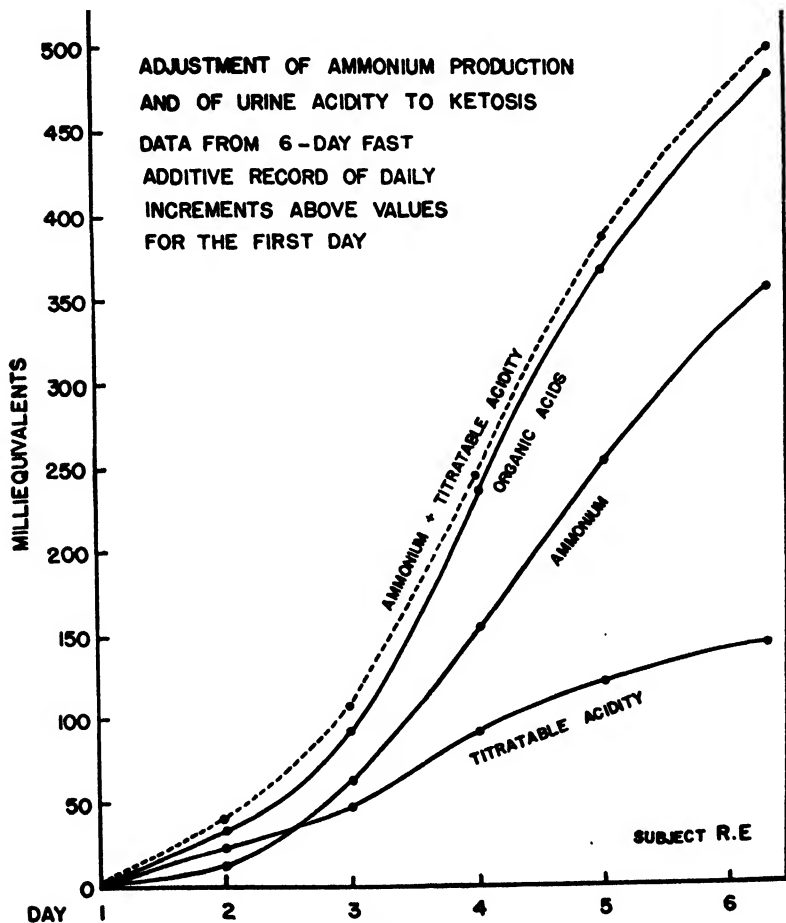measurements are of increments above the values found on the

ADJUSTMENT OF AMMONIUM PRODUCTION
AND OF URINE ACIDITY TO KETOSIS
DATA FROM 6-DAY FAST
ADDITIVE RECORD OF DAILY
INCREMENTS ABOVE VALUES
FOR THE FIRST DAY

AMMONIUM + TITRATABLE ACIDITY

ORGANIC ACIDS

AMMONIUM

TITRATABLE ACIDITY

SUBJECT R.E

MILLIEQUVALENTS

DAY

FIG. 7.

first day and are recorded additively across a 6-day fast.   So it
would seem that the ketone acids cannot be accused of carrying
fixed base into the urine during fasting.   Prevention of ketosis

will therefore not explain the large reduction of sodium outgo when glucose is given. Whatever the explanation of this sodium sparing effect of glucose, it obviously contributes importantly to maintenance of extracellular fluid.

Returning to figure 6, we find, then, that if we provide 100 gm. of glucose, the losses of extracellular water and of intracellular water which are incidental to the state of fasting will be reduced by about one-half. The chart suggests that by supplying intakes of protein and of sodium to cover the losses which are beyond the sparing effects of glucose we might gain complete conservation of the body fluids.

The results of the experiments recorded by figure 8 show you the failure of expectation of defense of body protein by intake. The daily rate of loss of protein when glucose was given was, for this subject, about 40 gm. When an approximately equivalent intake in the form of whole egg powder was provided along with glucose only a small further protection of body protein was gained. In other experiments in which the protein was supplied as casein or as wheat germ the outcome was the same. Evidently, under the circumstance of a sub-energy maintenance intake, ingested protein is oxidized in support of the energy metabolism. Our ignorance of the intrinsic terms of the metabolism of protein permits us to say that this is not a persuasive instance of the wisdom of the body. When glucose is given, protein catabolism is brought down to the physiological minimal and the energy metabolism is sustained by drawing on the body's abundant store of fat. Why cannot this excellent arrangement be continued and proffered protein be permitted to cover protein catabolism? When we learn more about the nitrogenous exchange than just its outside dimensions, the impertinence of this question will undoubtedly be revealed. The wonderful work of Schoenheimer has given us a start in that direction.

The outcome of these experiments makes clear the inutility of protein in a survival ration in which water intake must have priority over energy supply. A protein intake will not appreciably protect body protein and removal of the products of its

oxidation will greatly extend the renal water requirement. But now that means of obtaining water outside the ration have been
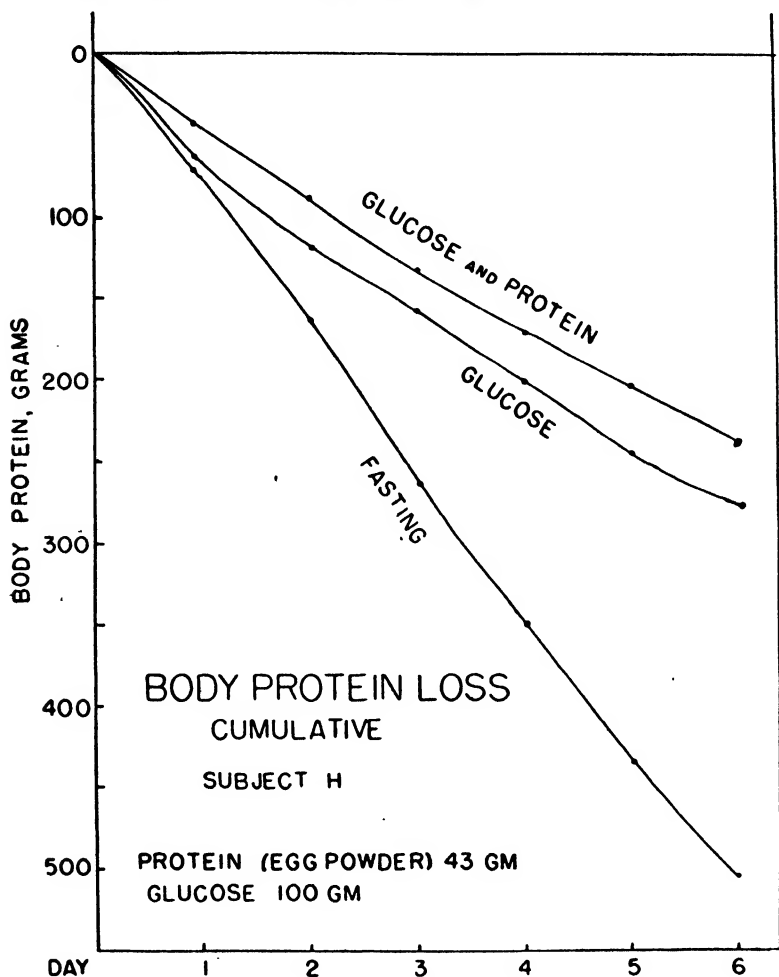
FIG. 8.

found, a much larger food intake can, of course, be provided. There is therefore need for further experimentation which will

tell us at what level of energy supply the physiologically minimal protein intake will be permitted to sustain body protein.   Dr. David Schwimmer and his group, who have studied the problem of emergency ration design very extensively at Welfare Island, have undertaken this important inquiry.   On the basis of a daily protein intake of 40 gm. they have found that nitrogen equilibrium is not approached until energy intake is raised to about 1800 calories.   This is considerably, as much as 30 to 40%, below estimated energy expenditure by their subjects.   This is unpublished information which Dr. Schwimmer has kindly permitted me to quote.

The data which this chart presents cast a shadow over the administration of protein hydrolysates parenterally unless caloric requirements can also be met.   The provision of an adequate calory intake parenterally is a large and difficult undertaking. It is probably not indicated unless there has been extensive depletion of body protein and fat.   So that in most clinical situations in which we undertake to sustain the body fluids we should be content with the extent of conservation of intracellular fluid gained by providing glucose.

Now, to consider the project of covering the loss of extracellular fluid which is beyond the sparing effect of glucose by providing an intake of sodium.   The results of four separate 6-day experiments with the same subject are recorded by figure 9.   The extent of reduction of sodium loss found for fasting when glucose is given is shown again.   It is interesting that this reduction is larger than was gained by providing a daily intake of sodium equivalent to the rate of loss during fasting.   When, however, the sodium chloride is given together with glucose, the deficit beyond the sparing effect of glucose alone is neatly repaired.   So we can, then, completely prevent the loss of extracellular fluid which is incidental to fasting by giving sodium chloride along with glucose.

Our experience with sea water led us to examine the effect of this very much smaller intake of the extracellular base sodium on the balance of the intracellular base potassium.   The average daily balances for sodium and potassium found in each of the

four experiments are shown by figure 10. The traditional un-
friendliness between sodium and potassium is evident. When
sodium chloride was given alone there was considerable reduction
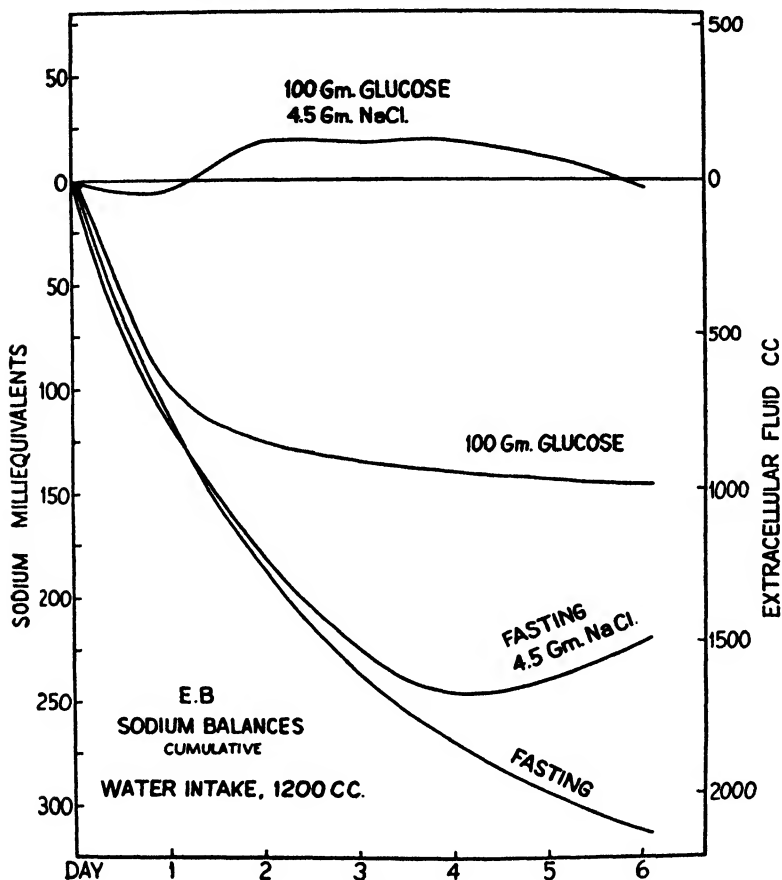

FIG. 9.

of sodium deficit but the loss of potassium was almost correspond-
ingly increased. An intake of glucose reduces the losses of both
ions. When sodium chloride is given along with glucose, sodium
is almost completely conserved but there is a large extension of

the loss of potassium. The implication of these data is that conservation of extracellular fluid gained by giving sodium will be at the cost of an increased loss of intracellular fluid. This would

FASTING · 100 Gm. GLUCOSE
WATER INTAKE 1200 cc.
4.5 Gm. NaCl. · 4.5 Gm. NaCl.

MILLIEQUIVALENTS PER 24 HOURS

SUBJECT E.B

SODIUM AND POTASSIUM BALANCES 6-DAY PERIODS
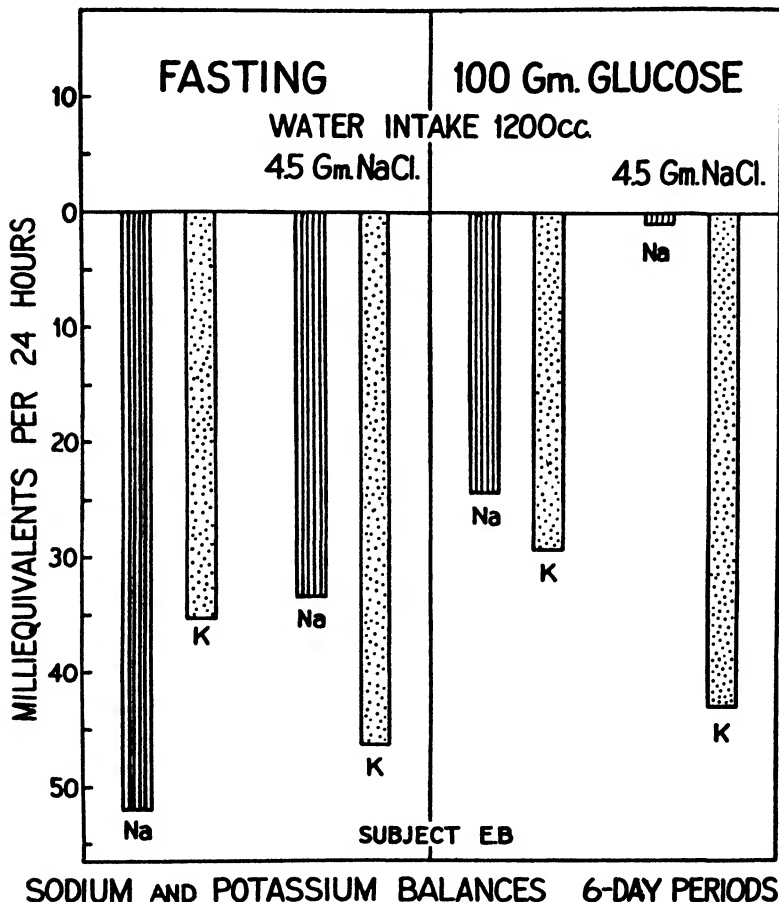
FIG. 10.

seem to be an unsound physiological bargain and so, apparently, we should be content with the conservation of extracellular fluid gained by glucose alone.

What the daily loss of body water amounts to when 100 gm. of glucose is provided with an ample water intake is shown in table 4. In relation to the initial water content of the body, which may be taken for a 70 Kg. individual as 14 liters of extracellular water and 50 liters of intracellular water, these losses may be regarded as unimportant unless their continuance is unusually prolonged. They will, however, be extended unless the obligatory expenditures of water by the body are completely covered by intake. So we have left for consideration the quantity of water which should be provided along 'with glucose in order to complete what may be

TABLE 4

Body Water Losses
Per 70 Kg. per day

Experiment	Extracellular water	Intracellular water	Total·
	cc.	*cc.*	*cc.*
Fasting	234	253	478
Glucose 100 gm.	120	146	266

Ample water intake.
Values average from 5 fasting and 3 glucose experiments.

called the physiological requirement in parenteral fluid therapy. Data which produce definition of this requirement are presented by the diagrams in figure 11.

One liter is a convenient and also quite liberal allowance for the insensible expenditure of water by a patient at rest in bed provided excessive vaporization of water is not produced by circumstances of disease or, as is frequently the case, by faulty control of the patient's immediate external environment.

The other section of the chart provides data for estimation of a suitable water allowance to the kidney. For a patient subsisting on 100 gm. of glucose, the daily load of solutes claiming removal in urine is one-half as large as is found for fasting and is only one-third of the load produced by a usual food intake. The urine volume required for removal of these several loads,

according to concentration of solutes in the urine, is shown by the ordinate. For a patient receiving only glucose, minimal volume at maximal concentration is about 300 cc. Since, however, water can be easily provided it is not necessary to demand maximal solute concentration. The kidney may be permitted to avoid the osmotic work which this requires and secrete urine at a usual concentration which can be taken as about 0.6 osmolar. At this
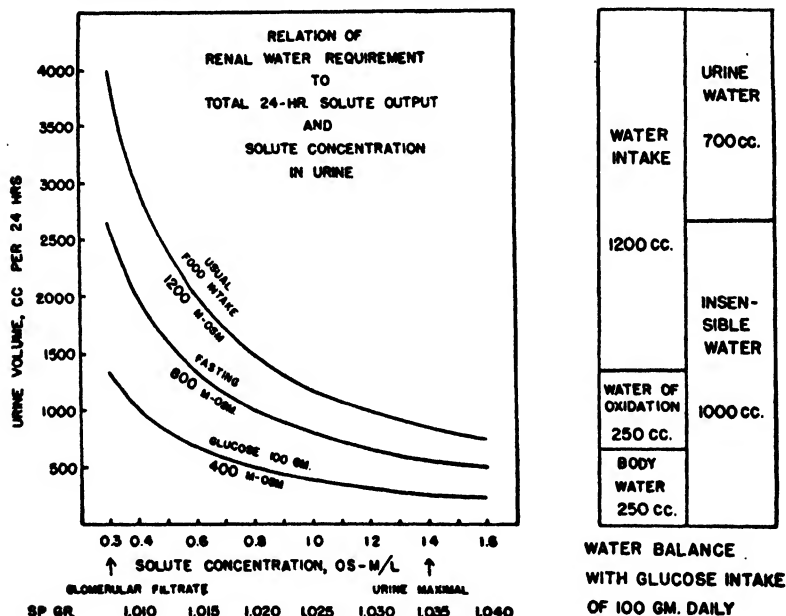
Fig. 11.

concentration the renal water requirement is something less than 700 cc. Total water expenditure will be covered to the extent of 250 cc. by water produced within the body by oxidation of body fat and protein and of the glucose intake. The body water loss beyond the conserving effect of glucose (table 4) provides another 250 cc. A water intake requirement of 1200 cc. is thus defined. If 100 gm. of glucose is given as a 5% solution a volume of 2 liters will be required. This will supply a large surplus of water. If

we wish to provide a margin for both water and glucose 1500 cc. of a 10% solution of glucose can be taken as a convenient statement of the physiological requirement in parenteral fluid therapy.

Losses of body fluids caused by pathological processes of dehydration will require replacement by a suitable solution of electrolytes. The services of glucose solution and of solutions of electrolytes are thus quite separate. We expect the body to spend the water provided as glucose solution but we wish the water given along with the electrolytes to be retained. This will not be the case unless obligatory expenditures are completely covered by glucose solution. It is also evident that the physiological requirement for expendable water will be stationary until oral intake is resumed, whereas the requirement for replacement of pathological losses of the body fluids by solutions of electrolytes should decline as deficits are repaired and processes of dehydration abate. Neglect of this consideration leads to overhydration.

It would seem to be appropriate to close this lecture on a note of admiration of the kidney. This chart provides an opportunity. These curves define a declining conservation of water expenditure as solute concentration in the urine is progressively increased. For instance, if we follow the 1200 milliosmol curve, urine volume required at the solute concentration of glomerular filtrate is 4 liters. By increasing concentration only as far as 0.6 osmolar, volume is reduced to 2 liters. To bring volume down to 1 liter, concentration must be carried all the way to 1.2 osmolar and beyond this point reduction of volume with further increase in concentration becomes nearly negligible. We are told that maximal concentration is determined by a limit to the kidney's ability to do osmotic work. This may be the case. But it would be more gracious and appreciative of meritorious performance to say that the end point of renal effort on this path of diminishing return for increasing work is chosen with a remarkably realistic discretion.

EARLY HISTORY OF THE HARVEY SOCIETY[1]

EUGENE F. DU BOIS

Professor of Physiology and Biophysics,
Cornell University Medical School

IT IS hard to realize that there was a time when there was no Harvey Society of New York and no Harvey Lectures. For the last forty-two years the Society, the Lectures and the annual publication have been such a firm foundation that we forget our predecessors who laid the cornerstone.

The original idea for the Harvey Society arose in the mind of Graham Lusk and received the immediate support of Samuel J. Meltzer. The plan was outlined to a group of kindred spirits and adopted on April 1, 1905, the 327th anniversary of Harvey's birth. This meeting was held in Dr. Lusk's home in the same room where the Society for Experimental Biology and Medicine had been founded two years previously. Those present were: Lusk, Meltzer, W. H. Park, E. K. Dunham, James Ewing, F. S. Lee, Christian Herter, Simon Flexner, George B. Wallace, Theodore C. Janeway, P. A. Levene, E. L. Opie and J. J. Abel of Baltimore. Two notable members of this group still take an active interest in the Society.

The second article of the Constitution, published from time to time but seldom read, states: "The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented."

It will be noted that these are the fields included in the preclinical years of our medical colleges. In 1905 lectures in the clinical fields were well covered by the Academy of Medicine. Indeed, there has always been close cooperation with the Academy

[1] An address delivered March 20, 1947, at a meeting of the Harvey Society celebrating the centennial of The New York Academy of Medicine.

of Medicine, made even closer by a formal affiliation in 1928. It is only natural that all of the lectures have been delivered in Hosack Hall.

At the time the Society was founded there was a rebirth of scientific clinical medicine and clinicians were beginning to employ the tools and strict methods of medical research. We were catching up with the leaders in Europe and in the early years one fourth of our lecturers were foreigners. Those were the days when you had to be a good linguist to understand what happened to the English language when it had a strong German, Scandinavian; French or Oxford flavor.

A large proportion of the lecturers have held their academic titles in the clinical departments but have been known for their work in the fundamental sciences. Fortunately we pay no attention to artificial barriers between university departments. A Professor of Medicine may be an eminent bacteriologist. A Professor of Pharmacology may be more of a physiologist than pharmacologist. The Harvey Society never chooses a lecturer for his title. He must be a scientist whose name is so much better than his title that the title is forgotten. No attention is paid to academic distinctions but an invitation to lecture is a recognition equivalent to or better than an honorary degree from a university.

Attempts have been made to classify our lecturers according to their official titles. May I suggest to all that you take down the 1929–1930 volume of the series and read once more the account by Graham Lusk of the first organization and the review of twenty-five years by Rufus Cole. Cole made an analysis of the first 220 lectures and found that 52 were by clinicians. Physiologists were second on the list and biochemists third. My own analysis of 136 lectures between 1930 and 1946 shows that there have been 21 physiologists, 29 clinicians (so-called) and 29 biochemists. You will note that biochemistry instead of being third is tied for first place. By the end of this season the clinicians will be leading once more but we made up for that by having this evening as our president a biochemist and as our lecturer a clinician who is distinguished for his work in biochemistry.

FACTORS AFFECTING THE KINDS AND QUANTITIES OF PENICILLIN PRODUCED BY MOLDS[1]

W. H. PETERSON

Professor of Biochemistry, University of Wisconsin, Madison

INTRODUCTION

PENICILLIN, like many other products, is essentially a war development. Although its great importance was foreshadowed in 1939, it was the pressure of war that brought its remarkable therapeutic properties into full relief and consequently intensified efforts to produce it in quantities adequate for the war needs. Following the dramatic demonstration of the therapeutic value of penicillin by the English workers, Florey, Chain, and associates (1, 2) in 1940 and 1941, the production of the antibiotic in quantity was turned over to the United States as the country that had available facilities and manpower equal to the task. The early developments in this country brought about through the efforts of the Office of Scientific Research and Development, several pharmaceutical houses, and the Northern Regional Research Laboratory, have been described in an interesting article by Coghill (3). Eventually mass production of penicillin became the responsibility of the Office of Production Research and Development, a division of the War Production Board. The full resources of the government were put behind the project and through the Defense Plant Corporation $20,000,000 were invested in 1943 and 1944 in the construction and equipment of plants located in many parts of the United States. Since much information was needed regarding the most suitable cultures, conditions of fermentation, and recovery of product, an intensive research program was started on these problems at several universities. I shall deal mainly with the work done at the University of Wisconsin. At the outset I should like to say that the

[1] Lecture delivered April 17, 1947.

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data I shall present have resulted from the efforts of many colleagues and assistants in the Departments of Biochemistry, Bacteriology, and Botany at our institution. As originally set up by OPRD, the penicillin project was limited to the fermentation aspects; and, although no such limitations now exist, we are still chiefly concerned with the metabolism of the mold and not with the recovery of the penicillin from the medium. The factors that affect the production of penicillin by the fungal cell are so numerous, complex, and challenging that they have fully occupied all our abilities and facilities.

The production of penicillin has many aspects in common with the agricultural production of cereals. In a certain sense it can be regarded as factory farming with a crop being harvested every day and a given acreage (a tank) yielding a crop every 3 to 4 days. This factory farming is a biological operation and requires the combined services of mycologists, bacteriologists, chemists, and engineers to run properly and efficiently. In fermentation work it is never safe to overlook biology as so often has been done by chemists and engineers, who frequently deal with a microorganism as if it were a chemical reagent and expect a response similar to that evoked in a chemical reaction. A more realistic approach takes the view that yeast, bacteria or molds—all living things—are being grown, and when proper conditions for growth and metabolism are maintained the products such as ethanol, butanol, and penicillin come as a natural consequence. Just as the successful farmer obtains the best crops by selection of suitable seed, application of proper fertilizer and careful cultivation, so the commercial producer of penicillin uses the highest-yielding strain of mold and aims to secure conditions that are most conducive to penicillin formation such as optimum aeration, pH, and nutrients. We may call this "factory farming," "biological engineering" or "industrial microbiology" as suits our fancy; but the fundamental principle to keep in mind is that we are dealing with a biological phenomenon. The better we understand the physiological processes involved, the more likely are we to obtain the kind and amounts of penicillin desired.

One of the most important factors that has brought about the enormous production of penicillin in the United States has been the use of high-yielding strains of molds. The Office of Production Research and Development was particularly active in promoting this type of research and set up projects for this purpose at Carnegie Institution, Cold Spring Harbor, and at the Universities of Minnesota, Stanford, and Wisconsin. Even before these projects were set up in 1943, very outstanding work had been done at the Northern Regional Research Laboratory, Peoria, Illinois. I think it is a reasonable estimate to say that probably 100,000 cultures were tested at these centers of research over the period 1943–45. Many of the cultures were new isolates from nature but the largest number were mutants obtained from spores treated with x-rays or ultraviolet radiation.

These isolates were put through a rough screening test (e.g., agar plates, small liquid fermentations), and the best of these were tested further in flask cultures. The most promising of the flask cultures were sent to the Northern Regional Research Laboratory and to the University of Wisconsin for further testing in tank fermentations, as these two places were the only ones equipped for such work.

The first culture of outstanding industrial importance uncovered by the research groups set up by the Office of Production Research and Development was X1612 (4, 5). It was obtained by x-ray treatment of *Penicillium chrysogenum*, No. 1951.B25, the Northern Regional Research Laboratory isolate, which was the highest penicillin producer in submerged fermentations then available. This work was done by Dr. M. Demerec of the Carnegie Institution, who sent it to the University of Minnesota for further testing in shaken flasks. It was sent to us with five other cultures for testing in tanks. While it had been as good as any culture in shaken flasks, its surpassing ability was first revealed in tank fermentation. The parent culture 1951.B25 under our conditions gave yields of 200 units per ml., but X1612 gave double that amount (400 units). Reports on its performance together

with transplants of the culture, were sent to all penicillin producers late in January, 1945. Throughout most of 1945, X1612 was the chief culture used in industry.

Work under OPRD contract was discontinued by our Botany Department on September 1, 1944, but Drs. M. P. Backus and J. F. Stauffer continued their search for better penicillin producers with funds from the University of Wisconsin Research Committee. They exposed spores of X1612 to ultraviolet radiation, and from several hundred cultures selected Q176 as distinctly superior to X1612. In the latter part of 1945 it was tested in tank fermentations and gave about 800 units of penicillin per ml., which was twice the yield from X1612 (6, 7, 8). When word of these high yields reached the producers, we were again besieged with requests for the culture, and in December 1945 and January 1946 transfers were sent to most of the producers in the United States and to many companies in England, France, Holland, Denmark, Sweden, and China.

By plating on an agar medium, Culture Q176 can be shown to consist of a very heterogeneous population. Dr. Backus has very kindly selected several of these types and prepared an agar plate showing the appearance of the different colonies (Fig. 1) grown on a honey peptone agar (6% honey, 1% Difco Bactopeptone, and 2% agar). The most abundant colonies (I and II), representing about 80 per cent of those that develop from spores show deep radial furrows and a tendency to wrinkle, especially in the center. A white marginal area devoid of spores and having a scalloped edge bounds the colony. Spores are moderate in number and vary in color from gray green to light green. A slow-growing colony much like the predominant type but smaller is shown at (III). A fourth colony (IV) shows less furrows and wrinkles and a smoother more uniform sporulation. A fifth colony (V) differs from the other four in being nearly devoid of spores. The most unusual type of colony (VI), appearing consistently but always in small numbers (2 to 5% of a Q176 population), is slow growing, strongly folded, has a peculiar pinkish or flesh color and is entirely devoid of spores for a long time. If left undisturbed

for from seven to twenty days, this colony develops sectors and localized areas where spores appear. Subcultures prepared by transferring spores from such regions give about the same number and proportion of variants as are found in the original parent
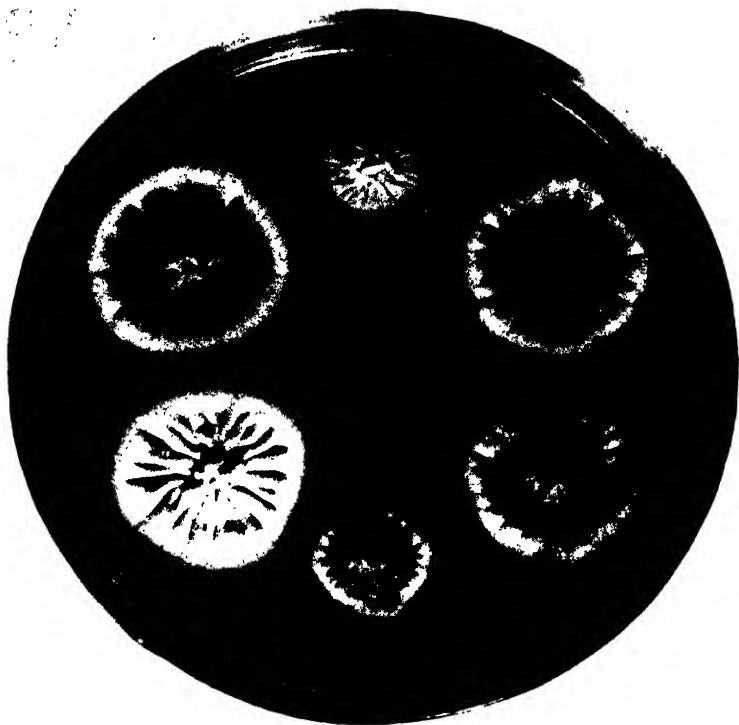
FIG. 1. *Penicillium chrysogenum,* Wis. Q176. This culture contains several morphological variants as can be seen from the size, appearance, and sporulation of the colonies.

culture, Q176. If hyphal tips of a young atypical colony of this type are transferred, the unusual appearance can be maintained indefinitely. This culture grows very slowly in shaken liquid medium but eventually, i.e., in 15–20 days, produces as much or

even more penicillin than the parent culture. While there are marked differences in the appearance of the strains, no cultural characteristics can be taken as an index of penicillin-producing ability. If any generalization were to be made, one might say that the strains showing the greatest variability in colony types have proved to be the best penicillin producers.

The relation of new cultures to penicillin production in the

FIG. 2: ADVENT OF NEW STRAINS AND COMMERCIAL
PRODUCTION OF PENICILLIN IN U.S.A.
AVERAGE MONTHLY PRODUCTION PER QUARTER
BILLIONS OF UNITS PER MONTH
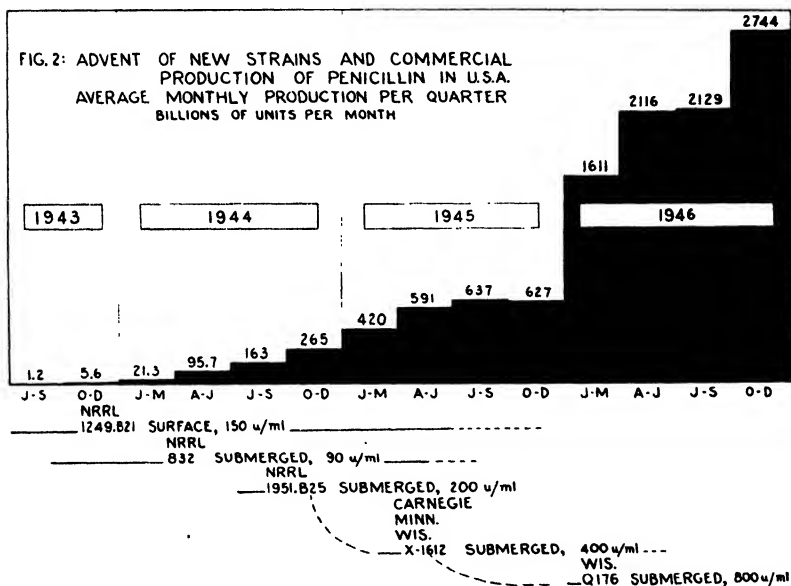
United States for the period 1943–1946 is shown in Figure 2 (9, 10). The figures printed above the blocks are the billions of units of penicillin produced per month during the given quarter. The cultures used in the industrial plants during these years are indicated by lines and other information below the blocks. In 1943 the monthly production was so small that it cannot be represented on this figure. In 1944 production rose from 21.3 billion units per month in the first quarter to 265 billion per month in the last quarter—a more than 10-fold increase. This was the anxious period during which it was firmly established that pro-

duction of penicillin in aerated tanks was possible and would soon free the industry from the laborious processing of millions of bottles of surface cultures.

Bottle production extended well into 1945, and during this time *Penicillium notatum,* No. 1249.B21, developed by the Northern Regional Research Laboratory was the strain in general use. It gave yields of about 150 Oxford units per ml. The first widely used submerged culture, *P. notatum* No. 832, was also developed by the Northern Regional Research Laboratory. It gave less than 100 units per ml., but a small tank of 832 broth yielded as much penicillin as 10,000 bottles of culture 1249.B21. An improved submerged culture, *P. chrysogenum,* NRRL 1951.B25, giving twice the yield of 832 was brought out in the middle of 1944 and was in use the rest of that year and for a few months in 1945.

It is noteworthy that in the quarter immediately following the discovery of culture Q176 the penicillin production in the United States was more than doubled. While one cannot attribute all of this increase to the use of Q176, there is no doubt that it was a most important factor in the sudden increase in production.

The figures for penicillin production in the United States have reached astronomical proportions, i.e., 2744 billion units per month. If the units are calculated to a weight basis on the assumption that 1 unit is equal to 0.6γ of penicillin G, the production per month for the last quarter of 1946 was about 3500 lbs. The market value of the penicillin at the plant for 1946 was about 100 million dollars, which makes penicillin second among the fermentation industries—alcohol products being first. So much for the economic aspects of penicillin. For the rest of the lecture I should like to discuss the scientific aspects of penicillin production. We have already considered cultures and will now take a look at apparatus, and finally at the metabolism of the mold.

APPARATUS FOR SUBMERGED FERMENTATION.

In submerged fermentations the mold mycelium grows as little pellets or short threads throughout the medium, while in surface fermentations it forms a thick mat which usually covers the whole

surface of the liquid. The simplest way of bringing about a submerged fermentation is to place the inoculated flask in a shaker that operates continuously and keeps the medium agitated. In a reciprocating shaker the mycelia grow in the form of small pellets, but in a higher-speed rotary shaker the growth appears as fine threads resembling a suspension in water of macerated filter paper.

Figure 3 shows a rotary type of shaker in which about 50 flasks
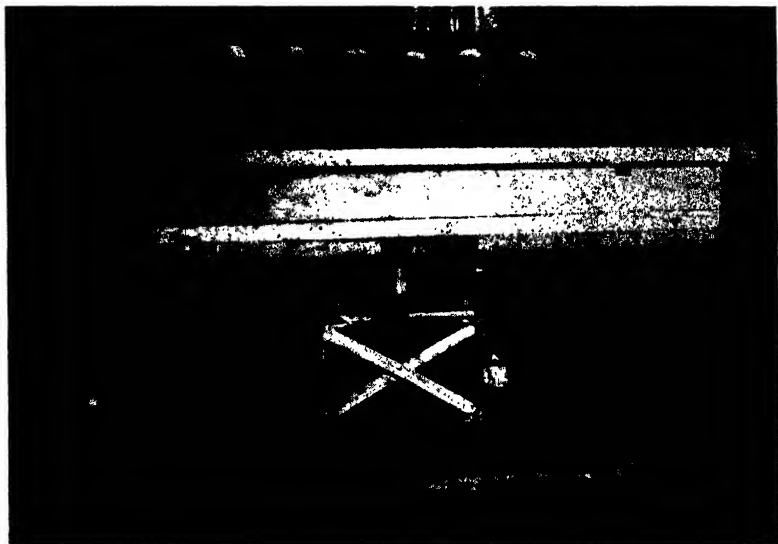
FIG. 3. Rotary flask shaker.

can be placed. A few flasks can be seen held in position at the back, but most of the places in the shaker are vacant. The shaker is supported on a central shaft and is kept in balance by legs of stiff rubber hose. It rotates on an eccentric that is off center about one-half inch and can be run at various speeds. A typical rate is 200 r.p.m. We also use a reciprocating shaker which has a back and forth displacement of 4 inches and is run at about 90 strokes per minute. It does not give as good aeration of the medium as the faster rotary shaker.

Our exploratory work is always done in shaken flasks, as hundreds of these can be run without much effort. From the results thus obtained, the most promising leads are followed in larger scale trials.

The next step in our experimental work has been done with pyrex glass jar fermenters (Fig. 4) in which the medium is aerated and agitated mechanically. The jar is placed in a metal frame and is closed by a cover to which are attached the various devices for aeration, agitation, and sampling. The fermenter illustrated contains no medium so it is easier to see the various devices attached to it. Air passes through a short pipe (A) packed with cotton which removes dust, bacteria, and mold spores from the air. The sterile air passes down through a small pipe and out through small holes in the ring of pipe or sparger at the bottom. The air escapes from the jar through a tube (B) which can be closed when necessary by a valve. The agitator (C) is a four-blade stainless steel propeller which is run by a pulley and belt. To increase the agitation of the liquid a metal baffle (D) is attached to the aerator tube. This breaks up the swirling motion of the liquid and produces a very turbulent agitation of the medium.

With much aeration and agitation a great deal of foam is produced. To keep down the foam, 3% octadecanol in lard oil is added from a reservoir (E) by an automatic device. One electrode of a circuit passes through the top of the jar to within about 4 inches of the surface of the medium (F). The aerator and other tubes serve as the second electrode. When the foam rises and touches the first electrode, the circuit is completed, the current passes through a relay which closes a valve, and air pressure forces some octadecanol-lard oil up through a tube into the jar on top of the foam. When the foam falls the circuit is broken and the flow of defoaming liquid ceases. Before we devised this automatic defoamer, we often found the greater part of the medium on the floor at almost any hour of the day or night.

When the medium has been placed in the jar and the cover attached, the entire apparatus is placed in a large autoclave and
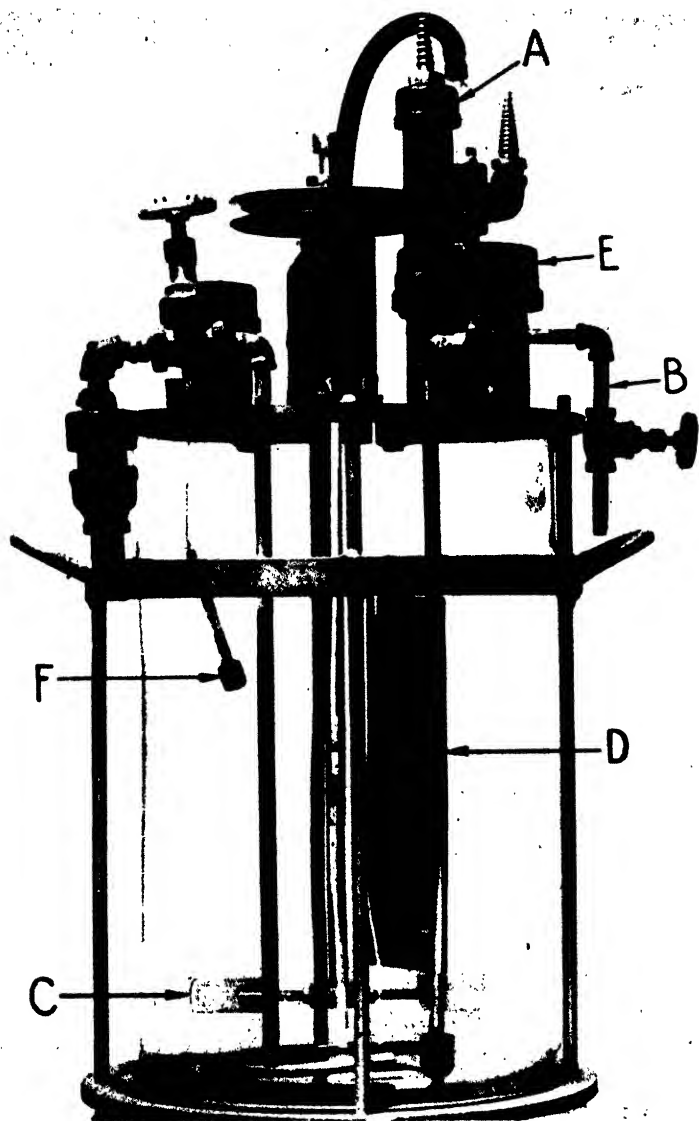
FIG. 4. Pyrex jar fermenter.

sterilized. After sterilization and inoculation, the fermenter is placed in a large water bath which is maintained at a constant temperature.

Figure 5 shows five of the fermenters in place in the water bath. The aeration is measured by a flow meter and the agitators are driven by a belt from the main shaft. In a typical fermentation one volume of air per volume of medium per minute and agitation
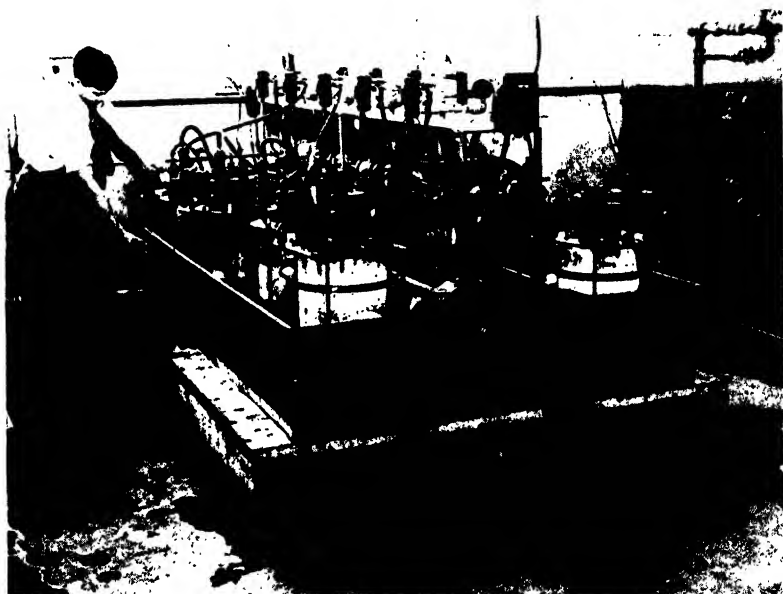

FIG. 5. Battery of jar fermenters in operation.

at the rate of 500 r.p.m. were used. The temperature of the bath is kept at 24° and the period of fermentation is about 72 hours. Ten fermenters can be run simultaneously; therefore, variations in medium, aeration and agitation can be made in a single experiment. These small fermenters have all the devices that are attached to large tanks. The period of fermentation is as short and the yields of penicillin are considerably higher than those obtained with our 100-gallon tanks. The jars of course enable

us to run many more tests at less cost than could be done with tanks. They have been in operation only about a year and would have been especially useful in the earlier work in testing cultures
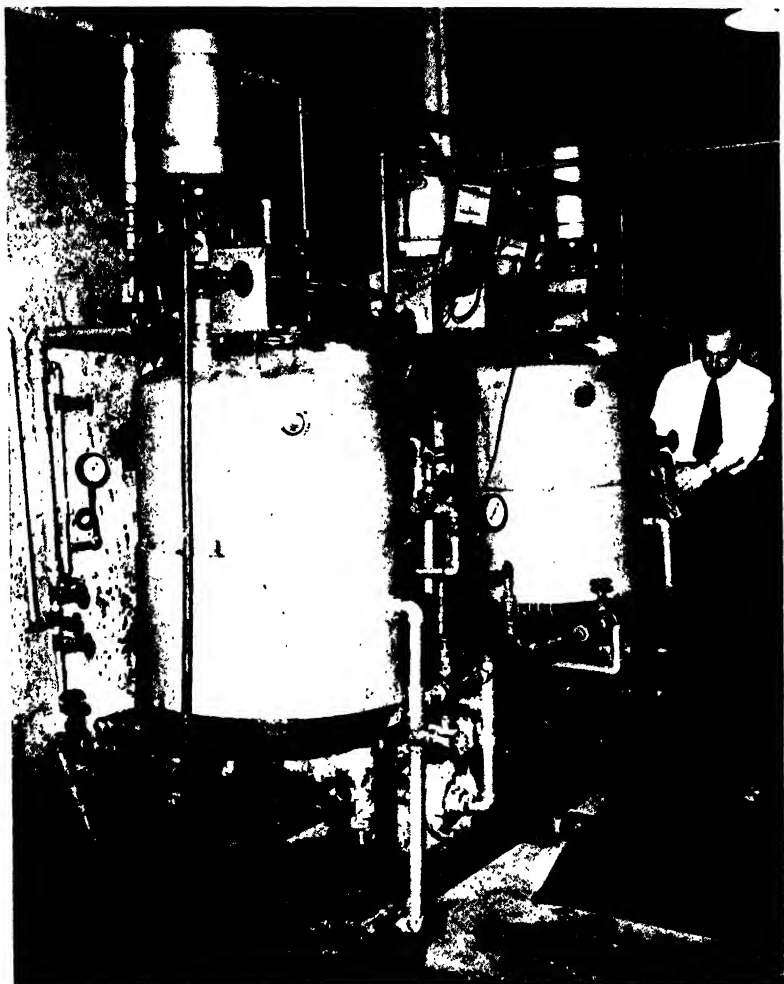
FIG. 6. Hundred-gallon fermenters and accessory equipment. For description of equipment and operation see Stefaniak *et al.* (7).

that gave promising results in flasks.   Flask results do not corre-late very well with tank results.

The next stage in the testing of our cultures or determining optimum conditions for penicillin production is with fermenta-tions in tanks of 100 gallons capacity (7).   Figure 6 is a photo-graph of two of these tanks in place.   They are equipped with flowmeters, air filters, aerators, agitators, and defoamers such as have already been described in connection with the jar fer-menters. ` During the war period when there was free exchange of information, our tanks gave yields higher than most industrial producers ·obtained, but not quite equal to those obtained by others.   Their outstanding usefulness is associated in our minds of course with the fact that they revealed the extraordinary peni-cillin-producing capacity of cultures X1612 and Q176.   Whether there were other better cultures in the vast number tested in flasks, but untried in tanks, no one knows.   Gray's lament about the unseen flower comes to mind:

> "Full many a flower is born to blush unseen
> And waste its sweetness on the desert air"

I haven't yet had the temerity to write a paraphrase of that lament for undiscovered penicillin cultures.

CHEMICAL CHANGES IN MEDIA

The next section of my lecture will deal with the metabolism of the mold under conditions of submerged growth.   A typical medium for penicillin production contains from 2 to 4 per cent lactose, 2 to 4 per cent corn steep solids, and 1 per cent calcium carbonate.   (Corn steep liquor is the concentrate of the water extract obtained in the industrial manufacture of starch, gluten, and other corn products.   It contains about 50 per cent solids.) The lactose provides a slowly fermentable carbohydrate and the corn steep solids supply carbohydrate, lactic acid, nitrogen com-pounds, inorganic elements, penicillin precursors, and an un-known number of other nutrients.   Dr. A. J. Moyer of the North-ern Regional Research Laboratory discovered its great usefulness

in promoting penicillin formation (3, 11), but in spite of much painstaking effort no one knows all that it contributes to the fermentation. It is both the delight and despair of everyone who works with it. When results don't turn out as expected, you can
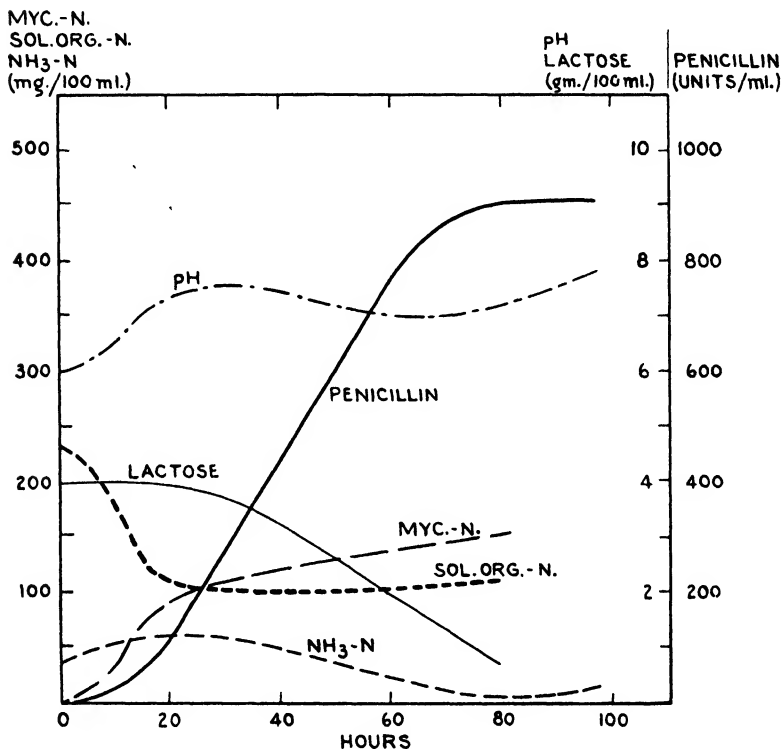
Fig. 7. Chemical changes in tank fermentation of lactose corn steep medium.

blame it on the corn steep liquor. That conclusion is a convenient one even though it is not very enlightening.

Figure 7 shows the characteristic chemical changes in a corn steep lactose medium produced by culture Q176 in tank fermentations (8). The penicillin fermentation can be conveniently divided into three phases (12). The first may be called a growth

phase, the second a stationary phase, and the third an autolytic phase.   In the first phase growth of mycelium is the prominent characteristic.   The mycelium is produced largely from the constituents of the corn steep, e.g., lactates, amino acids, and inorganic elements.   The carbon of the organic nitrogen compounds is used faster than the nitrogen and as a result NH_3-N increases, organic-N falls, and the pH of the medium rises.   In some runs a little lactose is used in the growth phase but in others, as in the example given, there is practically no utilization of the sugar. Penicillin formation is slow during the growth phase but becomes very prominent in the next phase.   In this period, pH, organic-N and mycelium-N remain approximately constant but lactose and NH_3-N decrease rapidly.   When these nutrients become low or exhausted, autolysis of mycelium with consequent rise in NH_3-N and pH sets in, formation of penicillin ceases and, in some fermentations, destruction of the antibiotic occurs.   For good penicillin production all the metabolic activities must be kept in proper balance.

Some of the chemical changes just described can be modified if suitable changes are made in the medium.   For example, if 1 per cent glucose is added to the lactose-corn steep medium a more available sugar is provided, utilization of amino acids as a source of carbon is avoided, and no increase in NH_3-N occurs.   Data for such a fermentation in 100-gallon tanks by culture Q176 are given in Figure 8.   An abundance of lactose was present even after 75 hours of fermentation and no autolytic phase set in up to this time.   There was no rise in pH, and NH_3-N and mycelium-N did not decline.   Penicillin formation continued steadily from about the 20th hour to the 73rd hour and reached the unusually high figure of 1,030 units per ml.   It is probable that if this fermentation had been allowed to run longer, the penicillin curve would have continued to rise—perhaps for another 12 to 24 hours.

The highest yield of penicillin, 1500 u/ml., which we have obtained in any fermentation has been secured in the jar fermenters containing a medium consisting of 1% glucose, 2.5% lactose, 4% corn steep solids, 1% calcium carbonate, 0.1% anhy-

drous sodium sulfate, and 0.15% ammonium acetate. The volume of medium was 14 liters. Aeration was at the rate of 0.64 liters of air per liter of medium per minute, agitation was 580 r.p.m., inoculum was 700 ml. of a 24-hour culture of Q176, and temperature of fermentation was 23° C. The ammonium acetate was
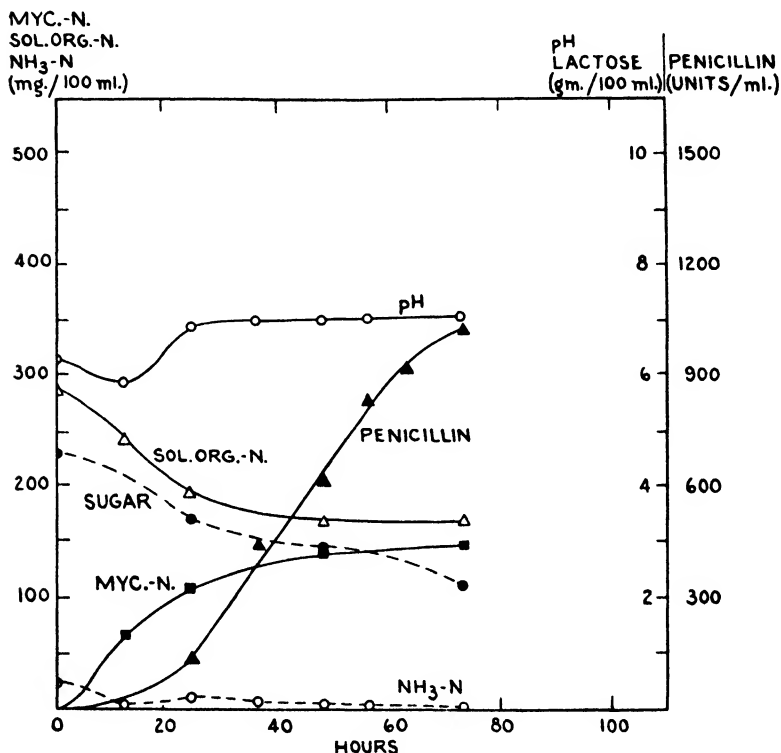

FIG. 8. Chemical changes in a glucose-lactose medium. Compare with figure 7 and note differences in pH, sugar, and mycelium-N curves.

added to increase the supply of readily available nitrogen as this corn steep liquor was low in NH₃-N. Sodium sulfate was added to insure an adequate supply of sulfur for building the thiazolidine part of the penicillin molecule.

The metabolic changes are given in Figure 9. In this fermen-

tation, utilization of ammonia and sugar began promptly and resulted in an unusually rapid building of mycelium.   Penicillin formation began early and continued markedly high up to the 80th hour, at which time 1,370 units per ml. had been produced. About this time indications of a lack of nutrients with a conse- quent rise in pH and slackening of penicillin production appeared.
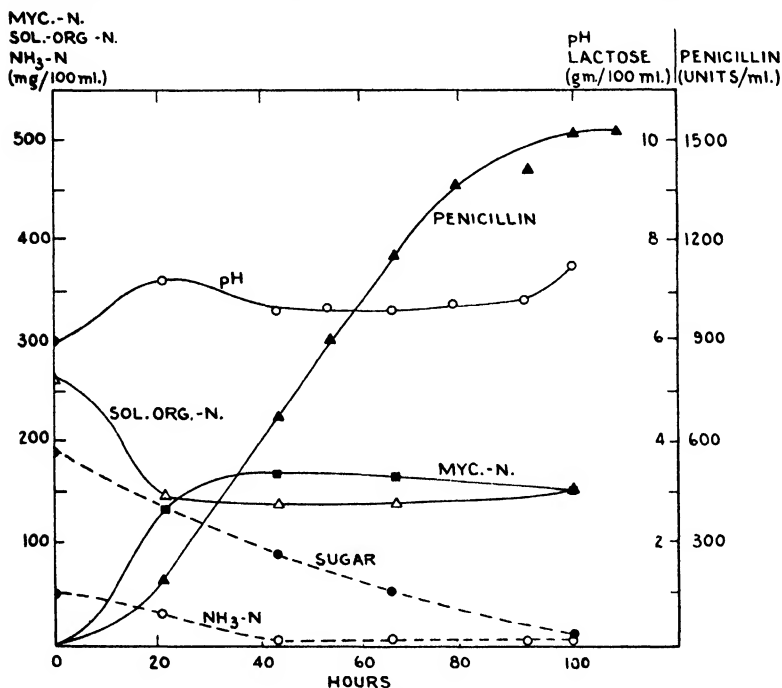
FIG. 9.   Chemical changes in jar fermentation producing highest yield of penicillin.

If a proper balance between nutrients and mycelium could be maintained, one might reasonably expect to get 2,000 units per ml.   Certainly there is no reason to believe that the penicillin- producing limit of the mycelium has been reached at 1,500 units per ml.   Chemical changes such as those reported in Figures 7 to 9 are speeded up by raising the temperature and are slowed down by decreasing the rate of aeration (7).

A study of the interrelation of the various nutrients to one another and to penicillin production is difficult in a corn steep medium because of the many unknown constituents contributed by the corn steep liquor. We have therefore spent a good deal of time devising a chemically defined medium that would give good yields of penicillin in shaken flasks (13). Such a medium
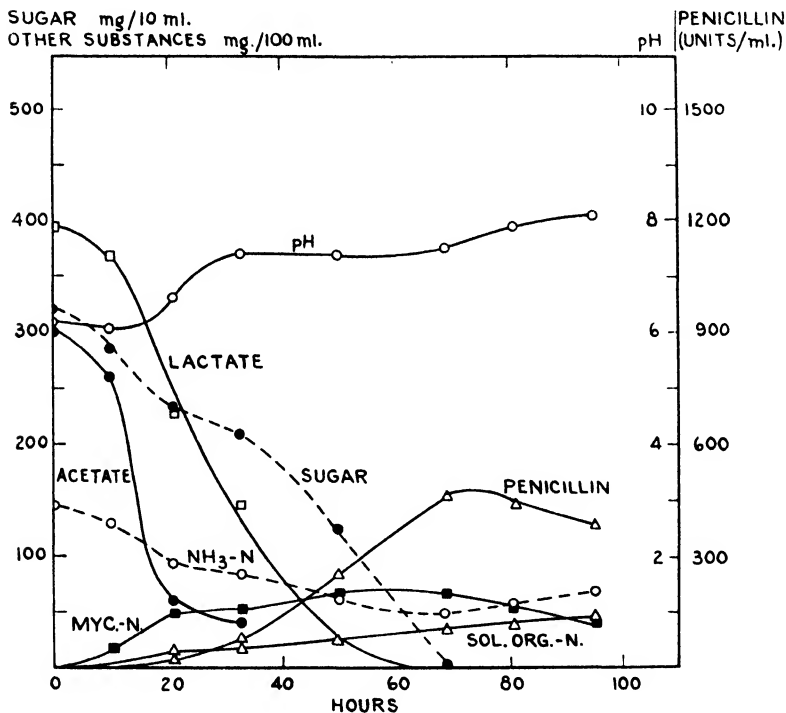
FIG. 10. Metabolism in a synthetic medium.

contains 0.75% glucose; 2.25% lactose; 0.3% ammonium acetate; 0.6% ammonium lactate, 0.05% phenylethylamine, 0.3% KH_2PO_4; 0.025% $MgSO_4 \cdot 7H_2O$; 0.002% $ZnSO_4 \cdot 7H_2O$; 0.005% $FeSO_4 \cdot 7H_2O$; 0.00025% $CuSO_4 \cdot 5H_2O$; 0.002% $MnSO_4 \cdot H_2O$; 0.05% Na_2SO_4.

The metabolism picture, Figure 10, shows that sugar, presuma-

bly glucose, and acetate were the chief carbon compounds used
for mycelial building during the first 20 hours.   In the next 20
hours sugar and lactate furnished most of the carbon.   If calcu-
lated to a molar basis, it becomes evident that ammonia was used
more slowly than the corresponding organic acids during these
two periods and hence caused a sharp rise in the pH curve.   From
the 40th to the 60th hours of fermentation, sugar utilization con-
tinued at a rapid rate, but mycelial-N, NH_3-N, and pH remained
relatively constant.   Penicillin appeared as soon as there was a
good amount of mycelium and a favorable pH.   It continued to
increase steadily until the sugar became exhausted and then fell
off.   The characteristic rise in NH_3-N and pH and fall in my-
celial-N set in immediately after the exhaustion of the sugar.
One is tempted to speculate whether the penicillin curve would
have continued to rise if additional sugar had been supplied.
Such an addition is obviously suggested by the data.

The yield of penicillin, 470 units per ml., is about two-thirds
that obtained with corn steep medium containing added pre-
cursor.   Without precursor the yield was about 300 units per ml.
The proper basis of comparison is with excess precursor added to
both media, since corn steep always supplies some precursor.

TYPES OF PENICILLIN

It is now well recognized that molds produce not one, but
several penicillins.   The relationship of five of these penicillins
to one another is indicated by the following structural formula
which has been proposed by the Committee on Medical Research,
OSRD, Washington and the Medical Research Council, London
(14) :
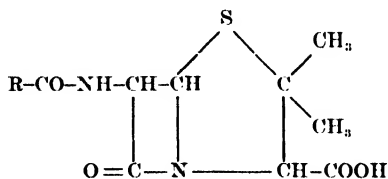
According to this formula all of the penicillins have a com-
mon lactam-thiazolidine structure, but differ from one another

in the side chain represented by the letter R. In G it is benzyl, $C_6H_5CH_2$; in X it is p-hydroxybenzyl, $HO \cdot C_6H_4CH_2$; in F it is Δ^2 pentenyl, $CH_3CH_2CH{=}CHCH_2$; in K it is heptyl, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2$; and in dihydro F it is amyl, $CH_3CH_2CH_2CH_2CH_2$. In most culture media you can greatly increase the yield of penicillin by adding a suitable precursor such as phenylacetic acid, in which case you will get not only a higher yield of penicillin, but a higher proportion of penicillin G. Strange as it may seem, the bottleneck in penicillin formation by the mold is not the synthesis of the lactam-thiazolidine part, but the making of the side chain.

Dr. O. K. Behrens of Eli Lilly and Company has obtained a large number of other penicillins from the culture broth by putting in suitable precursors. He discussed these new penicillins at the antibiotics meeting of the New York Academy of Sciences and again at Gibson Island, but because of war-time restrictions that still continue, a printed statement regarding them is not permissible. A full report on these penicillins will appear in the forthcoming monograph on the chemistry of penicillin sponsored by the American and British committees. One cannot view this array of penicillins without being impressed by the versatility and also by the non-discriminatory character of the mold cell.

The production of such a number of penicillins naturally raises the question, "What penicillin or penicillins does a mold make if it has to do the whole job?" Or to put it more directly, "What penicillins does a mold make when it is grown on a chemically defined medium without any precursor?" It is impossible to study this problem with a complex medium such as corn steep-lactose-salts. Corn steep contains so many compounds that perhaps it serves as precursor for a whole "flock" of penicillins.

A satisfactory answer to the problem will come as better methods for the determination of the several kinds of penicillin become available. These methods must be such that they can be applied to the broth as well as to the commercial product. At present in our research we use a combination of three different

methods for determining the components in broth.   These are microbiological, chromatographic, and extraction procedures.

From a survey of about 60 bacteria we found two that showed marked differences in their response to penicillins G, X, F, K, and dihydro F.   These bacteria are *Bacillus brevis* and an unidentified spore-forming lactic, called E.   Together with *Staph. aureus* they serve as a means of determining mixtures of three penicillins (15).

Figure 11 shows the response of *B. brevis* to the purest preparations of penicillins G, X, F, K, and dihydro F that we have been able to obtain from various laboratories and commercial producers.   The test depends on measuring inhibition of growth when penicillin is added to a liquid medium.   The effect is measured in galvanometer readings of the turbidity after about 15 hours incubation.   Since *B. brevis* is rather aerobic, the tubes are shaken continuously during the incubation period.   Maximum growth is indicated by a reading of about 35 and no growth is of course 100.   Units on the base line are all expressed in terms of penicillin G.   The curve for penicillin G is very steep, and complete inhibition of growth was obtained at about 0.025 units per ml.   The curve for X slopes a little more and ranges from no inhibition of growth at about 0.015 units per ml. to no growth at about 0.05 units.   There was some doubt about the purity of the sample of dihydro F, hence the question mark.   This curve is very close to the X curve and falls within the same range as the latter.   The F curve shows that *B. brevis* is much more resistant to this penicillin than to the other three.   The K curve being farthest to the right shows it to be the least effective of the five penicillins against *B. brevis*.

Similar curves which have been given elsewhere (15) are set up showing the effect of the five penicillins on organism E.   For this bacterium, X is the most potent penicillin; then come G, F, dihydro F, and K in order.   The range, 0 to 0.08 units per ml., is about the same as that for *B. brevis*.   There is but one curve for *Staph. aureus* since all penicillins are expressed as units of penicillin G.   Here the range is smaller, extending only from 0

to 0.025 units per ml. From the curves for the three organisms, activity ratios may be calculated and equations set up for calculating the composition of mixtures of penicillins. To calculate the composition of a mixture of three penicillins, the unitage is determined with each organism, and at the same time a set of
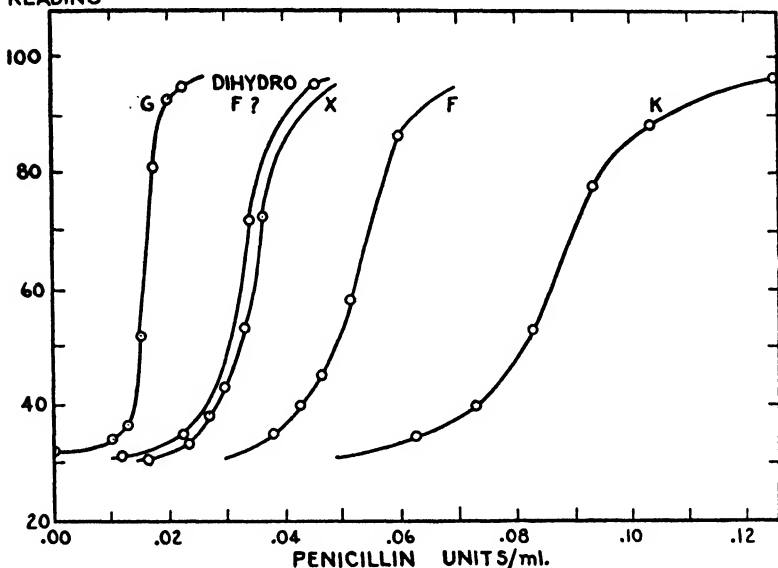

GALVANOMETER
READING

FIG. 11. Response of *Bacillus brevis* to five penicillins.

standards with each of the penicillins is run. The values found are entered in the three equations and then these are solved for G, X, and K.

Table I shows how well the method worked on known mixtures. The agreement between calculated and found was excellent with the first two mixtures consisting of G and K and G, X, and K respectively. Differences were more marked with the third mixture of G, F, and K—in two cases equaling about 10%. In a fourth test, K was added to a broth and analyzed to see if the added K could be recovered. The agreement between the found

and calculated values here were within 1%. We have used the method on a number of commercial penicillins whose components had been determined by other methods, and in general the agreement has been very good.

The most obvious limitation to the method is that the kinds of penicillin present must be known and the number of penicillins to be determined may not exceed three. Theoretically by means of a fourth organism a mixture of four penicillins could be ana-

TABLE I

Assays of Known Mixtures and Recovery of Penicillin Added to Fermentation Broth

Sample	Penicillins, % of total			
	G	X	F	K
1. Calculated	50	0	......	50
Found	49	< 1	......	50
2. Calculated	35	33	......	32
Found	33	36	......	31
3. Calculated	31	......	31	38
Found	40	......	32	28
4. Q176 broth	77	0	......	23
K added to give	37	0	......	63
Found	37	1	......	62

lyzed. Practically the errors in the assays are too great for the determination of that many components. While the method is not adequate by itself, it is very useful in conjunction with the other two methods.

Figure 12 shows the results obtained with an aqueous chromatogram run through a column of super filtrol and celite. Good separation of G and fair separation of X is obtained, but F and K come too close together for the data to be of much significance.

A 10-bulb extraction procedure based on the principle of Craig's (16, 17, 18) countercurrent extraction has given good results for the separation of K (Fig. 13). In this procedure 20%

butanol in chloroform was used as the solvent and the extraction
was done at pH 6.

By combining the three methods some tentative calculations can
be made as to the composition of the penicillins produced in a
synthetic medium. Assuming K, F, and dihydro F to be present,
the microbiological method gives the composition to be about 40%
K, 40% F, and 20% dihydro F. In corn steep medium, G is very
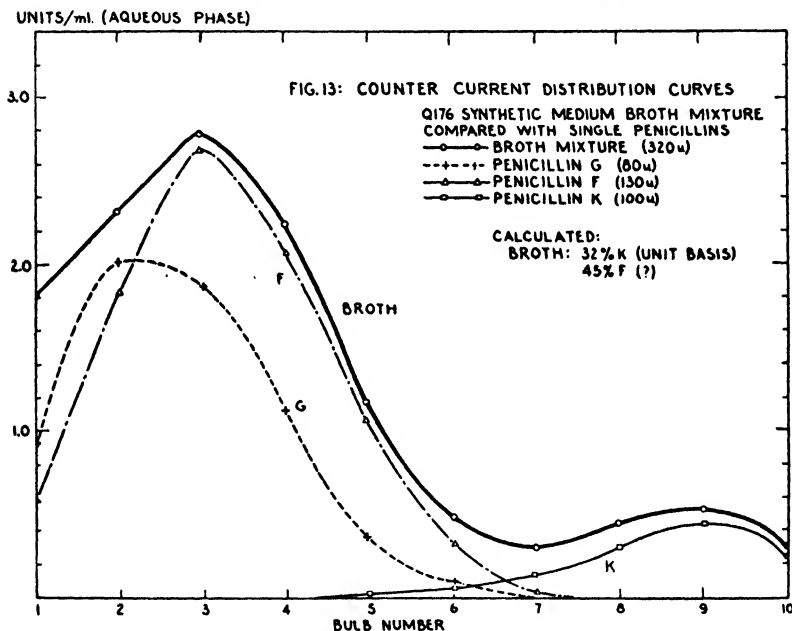prominent and presumably originates from phenyl precursors in

FIG. 12: AQUEOUS CHROMATOGRAPHY OF PENICILLINS
ON SUPER FILTROL - CELITE (1:1)

COLUMN: 14 X 130 MM
ELUENT: 0.05 M KH$_2$PO$_4$ pH 4.6

SAMPLES, 1.0 ml., CONTAINING:

PENICILLIN X	60 UNITS
PENICILLIN DIHYDRO F	65 UNITS
PENICILLIN F	70 UNITS
PENICILLIN K	80 UNITS
PENICILLIN G	167 UNITS
SYNTHETIC BROTH	170 UNITS
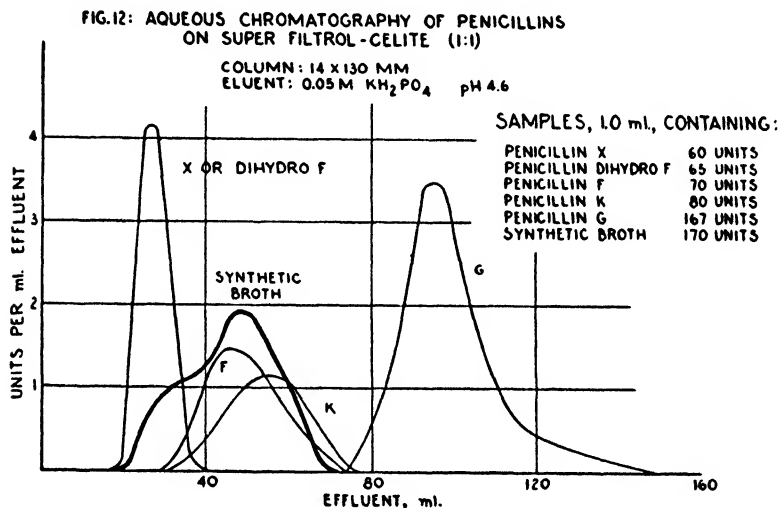
the medium. Perhaps we should say that the natural penicillins
are those that the mold makes on a synthetic medium, and those
arising from added precursors are induced or artificial products.
From this point of view F, dihydro F, and K are natural peni-
cillins and G, X, and Dr. Behren's compounds are artificial peni-
cillins. The absence of penicillins G and X cannot be attributed
to inability of the mold cell to synthesize the ring structures.
Mycelium grown on synthetic medium contained about 2% of
phenylalanine and tyrosine, each based on protein content, and
these amino acids contain the same ring structures as are found
in penicillins G and X respectively.

FUTURE DEVELOPMENTS

In closing, a few words may be ventured as to what appears to be ahead regarding factors affecting penicillin production. Probably many new penicillins will appear from the use of precursors, and some of these may prove even more useful than those we now have.

A study of the metabolism of the mold on a good synthetic

UNITS/ml. (AQUEOUS PHASE)

FIG.13: COUNTER CURRENT DISTRIBUTION CURVES
Q176 SYNTHETIC MEDIUM BROTH MIXTURE
COMPARED WITH SINGLE PENICILLINS
—o——o— BROTH MIXTURE (320u)
—·—·—·— PENICILLIN G (80u)
—△——△— PENICILLIN F (130u)
—o——o— PENICILLIN K (100u)

CALCULATED:
BROTH: 32% K (UNIT BASIS)
45% F (?)

BULB NUMBER

medium should show more clearly what influence amino acids and inorganic elements have on penicillin production. It should be possible to obtain an accurate picture of the carbon, phosphorus, and sulfur metabolism in such a medium.

A study of the metabolism of irradiation-induced mutants which lack the ability to synthesize a part of the penicillin molecule should give some insight into the mechanism by which the mold synthesizes the lactam-thiazolidine portion of the penicillin

molecule. As yet we know nothing about how this synthesis takes place.

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TYROSINE AND PHENYLALANINE METABOLISM IN INFANTS AND THE ROLE OF VITAMIN C[1]

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IN the course of an investigation of the physiologic creatinuria of infancy (1), it was observed that many premature infants fed cow's milk apparently excreted more preformed than total creatinine as determined by the Jaffe reaction (2). This surprising observation could only mean that unknown substances giving a falsely positive test for creatinine were present in the untreated urine and that these chromogens were destroyed in the process of converting creatine to creatinine. Acetone and acetoacetic acid which react in similar fashion were excluded as the interfering chromogens by qualitative tests.

This chance discovery deflected attention from the original study and initiated a new series of observations. The following questions required exploration: nature of the unknown urinary chromogens; their identification and methods of quantitative assay; conditions of occurrence; means of prevention and eradication; physiologic and clinical significance. Answers to some of these questions were made possible through the collaboration of my colleagues, Dr. H. H. Gordon, Dr. M. Dann and Miss E. Marples (3, 4, 5, 6).

NATURE OF URINARY CHROMOGENS

Separation and Differentiation. Differentiation of the urinary chromogens as substances distinct from creatinine was readily demonstrated (table 1).

Boiling the acidified (or untreated) urine destroyed the chromogen without affecting creatinine. Adsorption of creatinine by Lloyd's reagent and its insolubility in ether permitted quantita-

[1] Lecture delivered May 15, 1947.

tive separation of the chromogen in acidified urine. Although both substances gave a red color with the Jaffe reagent, the rate of color development was different, and the use of dinitrobenzoic acid and alkali, proposed by Benedict as a more specific test for creatinine (7), gave a yellow color with the chromogen instead of the purple rose color given by creatinine.

TABLE 1

Separation and Differentiation of Creatinine and Urinary Chromogens

	Creatinine	Chromogen
Heat (100° C.)*	No effect	Destroyed
Lloyd's Reagent (aluminum silicate)*	Adsorbed	Not adsorbed
Ether*	Insoluble	Soluble
Jaffe Reagent (alkaline picrate)	Red color†	Red color†
Benedict's Reagent (dinitrobenzoic acid + NaOH)	Rose color	Yellow color
Ferric Chloride (5%)*	Negative	Positive‡
Silver Lactate	No reduction	Reduction‡
Benedict's Sugar Reagent (qualitative)	No reduction	Reduction‡
Fiske-Subbarow Reagent (phosphomolybdic acid)	No reduction	Reduction‡
2,4-dinitrophenylhydrazine	No precipitate	Precipitate‡
Basic Lead Acetate	No precipitate	Precipitate‡

* Acidified urine.

† Rate of color development with creatinine is gradual, reaching and maintaining maximum by 8 to 10 minutes; color development with chromogen is prompt with 50% fading by 6 to 8 minutes.

‡ Positive tests point to a carbonyl derivative as a component of the chromogen.

The positive ferric chloride test, reduction with silver lactate, copper sulfate and phosphomolybdic acid, and the formation of a precipitate with 2,4-dinitrophenylhydrazine and basic lead acetate all pointed to a keto acid as a component of the unknown compounds.

Identification. The fact that the chromogens first appeared in the urine of premature infants when their diets were changed

from human milk to cow's milk mixtures of higher protein content (from 2.5 to 5 gm. or more per kg.) suggested that they might be intermediates of protein metabolism. The following qualitative tests for amino acids and other protein derivatives were therefore applied to chromogenic urines (table 2).

Positive xanthoproteic and Millon tests established the presence of phenyl or hydroxyphenyl rings in the unknown urinary compounds. Since phenylalanine, tyrosine and tryptophane are the only amino acids in natural proteins which contain the aromatic ring and since tests for the indole group, characteristic of tryptophane, were all negative, it seemed reasonable to infer that

TABLE 2

Qualitative Tests for Amino Acids and Other Protein Derivatives

Peptide Linkage		Indole Ring	
Biuret	Negative	Hopkins-Cole	
		Voisenet	
Free NH₂ and COOH Groups		Ehrlich	Negative
Ninhydrin	Negative	Nitroso-indole	
		Herter	
Phenyl or Hydroxyphenyl Ring		Obermayer	
Xanthoproteic	Positive		
Millon	Positive		

phenylalanine and tyrosine were the dietary precursors of the urinary intermediates. This inference was confirmed by feeding the pure amino acids (discussed later) and by chemical and spectroscopic identification of the urinary compounds. From chromogenic urines, p-hydroxyphenylpyruvic and l-p-hydroxyphenyllactic acids, the keto and oxy derivatives of tyrosine, were isolated and identified by elementary analysis, melting point, optical rotation and absorption bands, the substances conforming in all respects to the pure compounds. Concurrent analyses proved the virtual absence of other phenyl and hydroxyphenyl derivatives, including phenylalanine, phenylpyruvic and phenyllactic acids, tyrosine, homogentisic, hippuric and benzoic acids.[2]

[2] We are indebted to Dr. Hans Clarke and Dr. K. Dobriner for these analyses.

Quantitative Assay. The following methods were found suitable for quantitative assay of urines (table 3).

The methods listed in the table permitted measurement of total hydroxyphenyl and phenyl compounds, as well as separation of the ether-insoluble amino acids, tyrosine and phenylalanine, from their respective ether-soluble oxy and keto derivatives. The difference in turn of ether-soluble hydroxyphenyl compounds, determined by the Millon reaction (8, 9) and p-hydroxyphenylpy-

TABLE 3
METHODS FOR QUANTITATIVE ASSAY OF URINE

ruvic acid, determined by the reduction of phosphomolybdic acid (9), afforded a quantitative measure of p-hydroxyphenyllactic acid since the urinary output of other phenolic compounds remained constant and insignificant on diets of human or cow's milk.[3]

[3] Tyrosine as such, and phenylalanine as such, as well as its oxy and keto derivatives, appeared in the urine in significant amounts (over 50 mg. in 24 hours) only after ingestion of the pure amino acids. When this occurred, estimations of p-hydroxyphenylpyruvic acid tended to be falsely high in the

The routine dietary regimen in the premature nurseries consisted of feeding of heated human milk or cow's milk mixtures with supplements of vitamins A and D (20 drops of percomorph oil daily). Early in the study, a number of infants also received vitamin C as l-ascorbic acid in daily dosages of 10 or 20 mg. The following facts soon became evident.

Infants fed human milk, low in protein (3 gm. or less per kg. per day), never exhibited hydroxyphenyluria. Millon-reacting substances were invariably absent by qualitative tests of casual specimens examined routinely over a six-month period, and by quantitative assay of 24 hourly specimens they never totalled more than 50 mg. of tyrosine equivalent in 24 hours.

The appearance of hydroxyphenyl compounds (p-hydroxyphenylpyruvic and p-hydroxyphenyllactic acids) in the urine always coincided with shifts to diets of higher protein content (4 gm. or more per kg. per day). On these diets, however, exhibition of hydroxyphenyluria was irregular and its magnitude and duration, variable. When these diets were devoid of vitamin C, exhibition of the metabolic defect was consistent; on comparable diets with added vitamin C, it was irregular and inconstant.

Full term infants on equivalent or even higher protein diets, in contrast to premature infants, rarely excreted tyrosine intermediates in their urine.

These observations suggested more detailed study of the following variables: level of protein intake; maturity of the infant; role of accessory food factors, especially vitamin C.

Color, sex, birth weight, and age and weight at time of observa-

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presence of coexisting excretion of phenylpyruvic acid and estimations of phenyllactic acid were wholly erroneous with coexisting excretion of p-hydroxyphenyllactic acid. Even in the feeding observations with pure amino acids, other phenolic compounds, such as homogentisic acid, dihydroxyphenylalanine and melanin, phenaceturic acid, glucuronides, p-cresols and ethereal sulfates, were always absent by qualitative tests, absence of darkening of the alkalinized urine on standing, and unchanged excretion rate of conjugated phenols.

tions were readily excluded as factors of pathogenetic significance. White and colored, male and female, small and large, and young and old premature infants responded in similar fashion to equivalent dietary regimens.

Role of Dietary Protein. The dependence of hydroxyphenyluria on the level of protein intake is illustrated in figure 1.

Tyrosine intermediates were absent from the urine of the three premature infants while on feedings of human milk containing
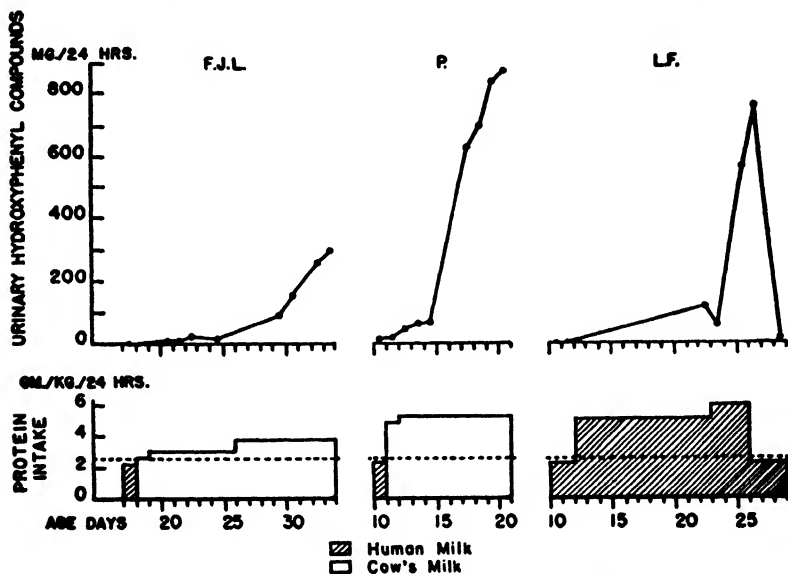

FIG. 1. Relation of protein intake to onset of hydroxyphenyluria.

less than 2.5 gm. of protein per kg. per day. Following an increase in dietary protein above a threshold level of approximately 4 gm. per kg., the urinary output of Millon-reacting substances rose sharply to peak levels after varying periods on the high protein diets. In one infant (L.F.), replacement in isocaloric amounts of casec by dextrimaltose in his feedings of human milk and the resultant drop in protein to below 2.5 gm. per kg. was accompanied by a prompt subsidence of hydroxyphenyluria.

The next figure shows even more graphically the dependence

of the metabolic defect on the level of dietary protein and more especially on the aromatic amino acid intake.

This premature infant received cow's and human milk of varying protein content (from 2.5 to 5.5 gm. per kg.) and correspondingly varying intakes of phenylalanine and tyrosine (from 0.65 to 2.0 gm. in 24 hours, expressed as tyrosine)[4] in 9 successive
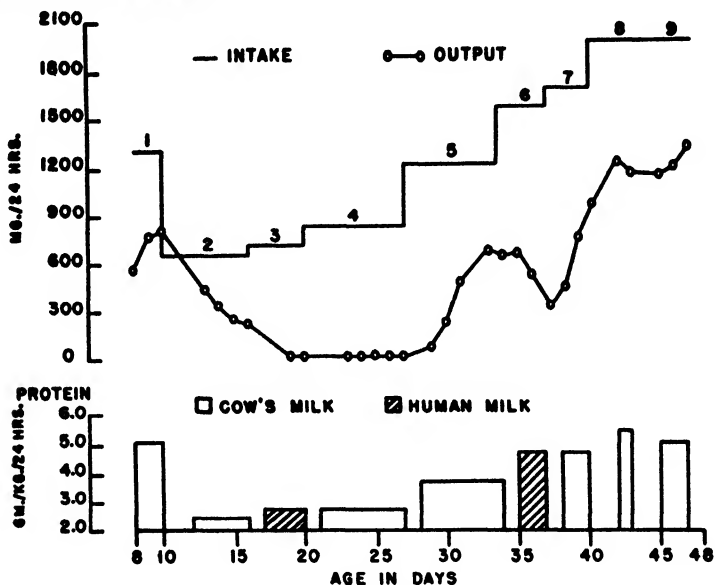
HYDROXYPHENYL COMPOUNDS

FIG. 2. Effect of protein and aromatic amino acid intake on hydroxyphenyluria.

periods totaling 40 days. A striking parallelism is noted between the level of aromatic amino acid intake and the magnitude of hydroxyphenyluria. Tyrosine intermediates were absent from the urine in the periods of low intake (periods 2, 3, 4) and present in the periods of higher intake, whether the feedings consisted of cow's milk (periods 1, 5, 7, 8, 9) or evaporated skimmed hu-

[4] The phenylalanine and tyrosine content of human and cow's milk was calculated from the figures given by Williamson (15).

man milk.[5] The excretion lag following dietary shifts and the brevity of individual periods precluded quantitative correlation of the threshold of aromatic amino acid intake and the onset of hydroxyphenyluria.

The excretion rate of intermediates was as much a function of the duration of high protein feedings as the actual level of protein intake above threshold levels (figure 3).
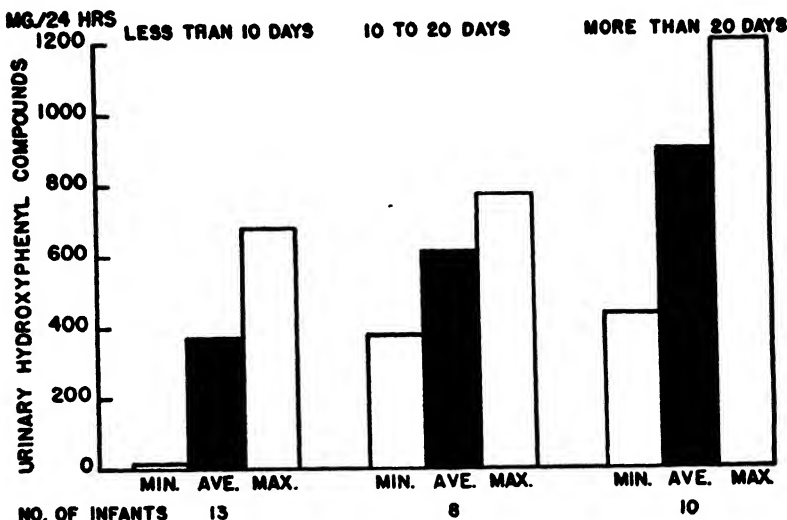

FIG. 3.   Effect of duration of high protein diets on hydroxyphenyluria.

The figure is arranged to show the range and average excretion of total hydroxyphenyl compounds by premature infants on comparable high protein diets for less than 10 days, for 10 to 20 days, and for longer than 20 days.   The levels of excretion varied directly with the duration of feedings, the average values for the three periods being, respectively, 370, 615 and 900 mg. in 24 hours.   A similar correlation held for individual infants.   In

⁵ That human milk did not contain accessory substances capable of ameliorating the defect is demonstrated by L.F.' (figure 1), by period 6 (figure 2), and by unpublished experiments by Dr. M. Dann in vitamin C–deficient guinea pigs fed unprocessed human milk.

every instance, the level of output rose progressively with prolongation of high protein feedings and persisted at high levels for the duration of observations, at times for as long as 2 to 3 months. The onset of hydroxyphenyluria, the time span required to reach peak levels, and maximal values attained, varied however for individual infants on equivalent protein and vitamin C–deficient intakes.

The total hydroxyphenyl compounds excreted by all premature infants (23 in number) on these diets for one week or longer reached peak levels ranging from 300 to 1385 mg. of tyrosine equivalent in 24 hours. The output of the keto derivative, p-hydroxyphenylpyruvic acid, ranged between 60 and 450 mg. in 24 hours with an average of 28 per cent for all the infants. The remaining 72 per cent represented p-hydroxyphenyllactic acid and minimal amounts of tyrosine (less than 50 mg. in 24 hours). These figures contrast with those reported for healthy adults: 150 to 230 mg. (ave. 195 mg.) of total urinary hydroxyphenyl compounds; 73 to 100 mg. (ave. 82 mg.) of the keto derivative per 24 hours (16).

Maturity of Infants. Although the quantitative degree of immaturity seemingly was not a determining factor in the exhibition of the metabolic aberration so long as the infant was prematurely born, full fetal maturity of itself was of great importance in pathogenesis. Only one of 12 full-term infants receiving cow's milk, high in protein, exhibited appreciable hydroxyphenyluria and this infant was the smallest in the series (birth weight, 2.53 kg.). Besides, more than 50 casual specimens of urine from full-term infants receiving cow's milk in the obstetrical nurseries responded negatively to the qualitative Millon test except for one infant with severe jaundice and congenital hepatic cirrhosis.

The capacity of full-term infants to handle the aromatic amino acids contained in their customary feedings of cow's milk without excreting intermediates in their urine proved to be limited. Even their tolerance could be exceeded by the ingestion of phenylalanine and tyrosine in pure form.

ARTIFICIAL INDUCTION OF HYDROXYPHENYLURIA

In both premature and full-term infants, the ingestion of either d,l-phenylalanine or l-tyrosine in a single dose of from 0.2 to 2.0 gm. per kg. precipitated the onset of, or accentuated an already existing hydroxyphenyluria (figure 4).

In accord with their usual behavior, the full-term infants did not excrete tyrosine intermediates on fore-period diets of cow's
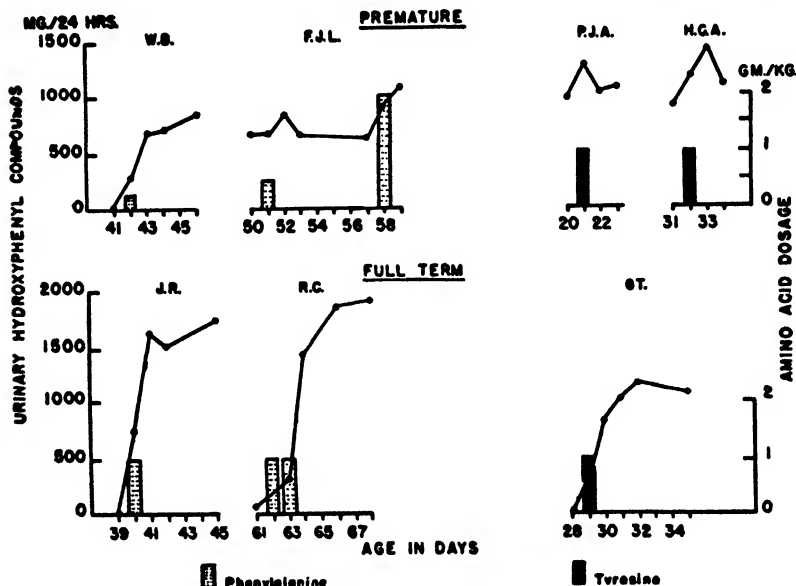

FIG. 4. Effect of a single dose of amino acids on hydroxyphenyluria in premature and full-term infants.

milk, high in protein, in contrast to three of the four premature infants whose initial outputs had already reached high levels on equivalent control diets. The response of both groups of infants to the ingestion of the amino acids was however similar; namely, the prompt onset of hydroxyphenyluria, when absent in the fore-periods, and its accentuation, when previously present.

The ingestion by full-term infants of either amino acid in one large dose of 1 gm. per kg. was sufficient to disrupt the enzymatic

mechanisms responsible for their degradation whereas the recurrent ingestion of slowly absorbed, divided doses in smaller, daily amounts as components of cow's milk protein did not flood the cells to the point of precipitating the defect.

The response of the infants, premature and full-term, to repeated ingestion of the amino acids in daily dosages of from 0.2 to 2.0 gm. per kg. for 4 or 5 successive days was in every way similar to that following single dosage except that excretion rates,
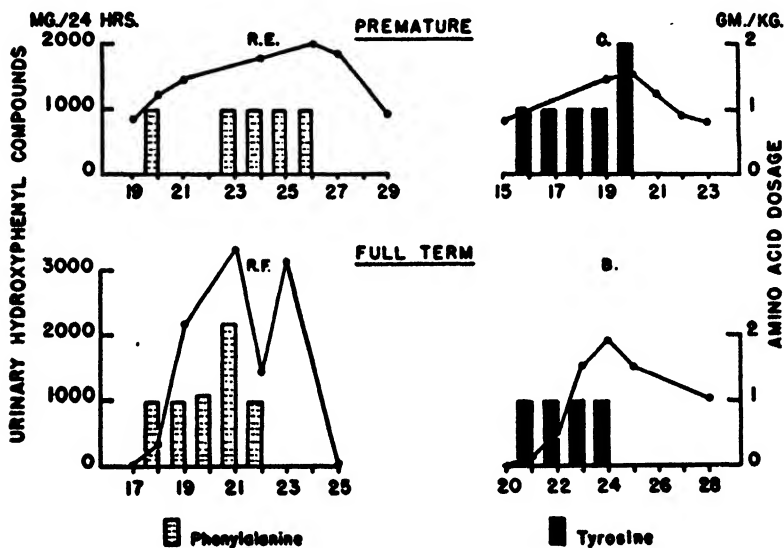

Fig. 5. Effect of repeated dosage of amino acids on hydroxyphenyluria in premature and full-term infants.

as might be expected, reached higher peaks in the test periods (figure 5).

Despite the expected absence of tyrosine derivatives in the urine of the full-term infants in control periods, their excretion levels in the test periods following both amino acids in similar dosage were higher than in the premature infants, again pointing to the quantitative nature of the difference in behavior of the two groups of infants.

It is also significant that excretion rates following phenylalanine exceeded those of tyrosine in both groups of infants. The higher urinary output following phenylalanine ingestion is probably explained by its more complete absorption from the alimentary tract, by its ready *in vivo* conversion to tyrosine (discussed later), and by the greater economy with which the animal organism handles absorbed tyrosine (17, 18, 19, 20). Analyses of stools showed that fecal loss of phenylalanine remained negligible following its ingestion in pure form in contrast to fecal losses of around 20 per cent of test doses of tyrosine in comparable amounts (0.5 gm. per kg. on each of 4 successive days) (6).

Partition of Urinary Hydroxyphenyl and Phenyl Compounds. The presentation to this point has been focussed on the excretion of total hydroxyphenyl compounds with only brief mention of individual fractions. It was stated that when premature infants exhibited the defect spontaneously, the urinary hydroxyphenyl compounds consisted of large amounts of p-hydroxyphenyllactic acid, lesser but substantial amounts of p-hydroxyphenylpyruvic acid, and traces of tyrosine. Other hydroxyphenyl derivatives were absent from the urines.

Ingestion of the pure amino acids, d,l-phenylalanine and l-tyrosine, not only raised the output of the oxy and keto acids of tyrosine but it provoked the appearance in large amounts of other derivatives: tyrosine, following ingestion of both amino acids; phenylalanine and its oxy and keto acids, following phenylalanine ingestion.

Partition of these constitutents under conditions of spontaneous exhibition, and following tyrosine and phenylalanine ingestion is shown in figure 6.

The results of the three sets of observations are charted in terms of average excretions in mg. per 24 hours on the left and as percentages, on the right side of the figure. Hydroxyphenyl compounds are recorded above the base line; phenyl compounds, below the base line.

Reference has been made to the relative contribution of each of the three hydroxyphenyl compounds to total hydroxyphenyl-

uria and the virtual absence of phenyluria[6] under conditions of spontaneous exhibition of the defect.   The ingestion of l-tyrosine led to the excretion in substantial amounts of the amino acid itself and to a heightened output of its keto and oxy acids.   Phenyluria remained negligible.   Following ingestion of d,l-phenylalanine in comparable dosage, both hydroxyphenyluria and phenyluria attained maximal values.   The percentage excretion
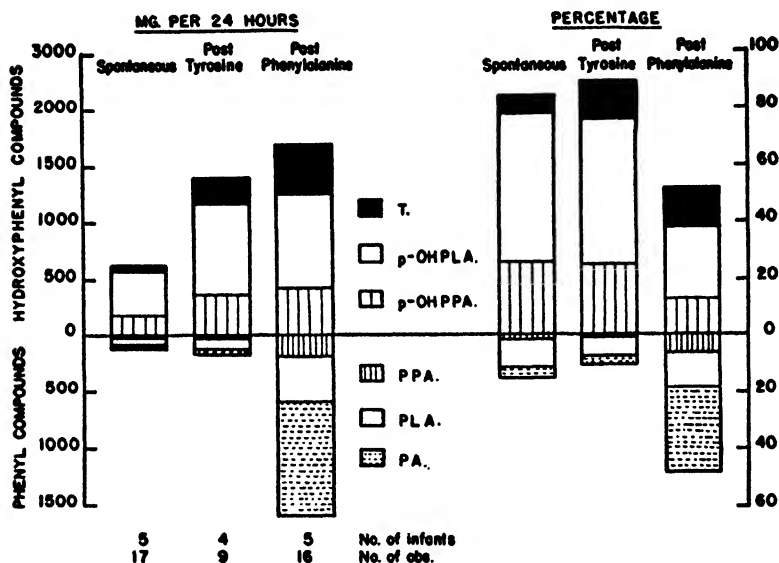

FIG. 6.   Partition of urinary intermediates.

of extra tyrosine or phenylalanine ingested in pure form ranged from 43 to 83 per cent in all the feeding observations.   Table 4 summarizes the results after ingestion of daily doses of 1 gm. per kg. of the latter amino acid.

The percentage of ingested phenylalanine excreted as urinary intermediates rose from 26 on the first day to 83 per cent of the

[6] The falsely high values for phenyllactic acid in the presence of p-hydroxyphenyllactic acid has been previously mentioned (footnote 3).   Omission of this fraction from the partition sheet would in no way invalidate interpretation of the results (table 4).

test dose on the fourth day of ingestion.   Special attention is directed to the mounting excretion rates of the amino acids themselves and to the persistently low excretion rate of phenylpyruvic acid with continuing ingestion of phenylalanine.

An explanation for the higher magnitude of hydroxyphenyluria following phenylalanine ingestion has been offered in the preceding section.   The high urinary output of phenyl compounds,[6] and especially of phenylalanine itself is undoubtedly attributable to simple overflow and urinary leakage (as in the

TABLE 4

Phenyl and Hydroxyphenyl Compounds in the Urine After Successive Daily Doses of 1.0 gm. per kg. of d,l-Phenylalanine*

Day of amino acid ingestion	No. of observations	% of ingested dose excreted as:				Total % of ingested dose excreted‡
		Phenyl- pyruvic acid	Phenyl- alanine	p-hydroxy- phenylpy- ruvic and lactic acids†	Tyro- sine	
First	8	1.7	13.9	8.6	1.7	25.9
Second	8	2.8	17.7	27.1	8.9	56.5
Third	5	1.9	17.3	32.8	11.7	63.7
Fourth	5	3.3	23.2	40.6	15.9	83.0

* Vitamin C-free diets.

† These two substances are grouped together for simplification.

‡ The figures represent minimal percentages since the output of phenyl-lactic acid (footnote 6) was omitted from the calculations.

feeding observations with tyrosine, figure 6) and to the fact that the racemic form, d(+), l(−)-phenylalanine, was given.   The available evidence indicates that the *in vivo* conversion of the unnatural enantiomorph, d(+), to its natural isomer, l(−), by d-amino acid deaminases involves intermediary deamination to the corresponding keto acid (21, 22, 23, 24, 25, 26) and a consequent reduction in metabolizability and utilization (27, 28).[7]

[7] This concept would not hold if a recent report indicating that d(+)-phenylalanine is quantitatively converted to its natural isomer by the growing rat (29) applies to the human infant.

Of special significance was the excretory response of phenyl-
alanine to tyrosine ingestion (upper part of figure 7) and of
tyrosine to phenylalanine ingestion (lower part). Whereas the
urinary excretion of phenylalanine (and phenylpyruvic acid,
figure 6) remained negligible with the ingestion of large amounts
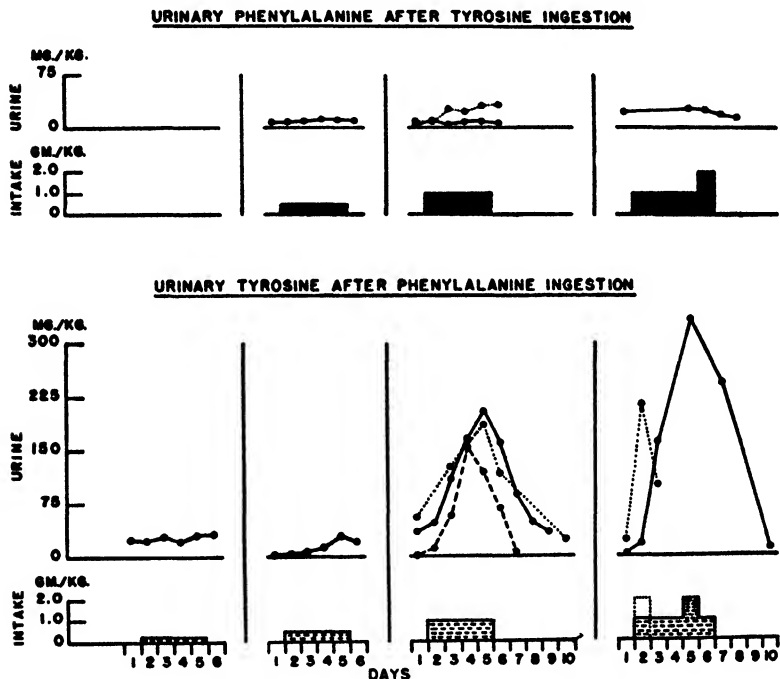of tyrosine (up to 2 gm. per kg.), the output of tyrosine (and

FIG. 7.

hydroxyphenyl derivatives, figure 6) reached high levels with
the ingestion of the same amounts of phenylalanine. In fact, in
all infants receiving 1 gm. or more of d,l-phenylalanine per day
for 4 days, the output of tyrosine was so high as to exceed its
solubility in urine, and the voided urine contained large numbers
of crystals of l-tyrosine.[8]

[8] Identification was made in Dr. Du Vigneaud's laboratory by elementary
analysis and optical rotation.

The results indicate that healthy premature and full-term infants are able to convert d,l-phenylalanine to l-tyrosine and that this conversion is an irreversible process in the living human organism.   They confirm and extend the conclusions derived from feeding (17, 30, 31, 32, 33, 34, 35), liver perfusion (36, 37) and *in vitro* experiments (38, 39) in animals, and human observations in normal adults (40, 41, 42, 43) and in patients with such inborn errors of metabolism as phenylketonuria (27, 44), alcaptonuria (45, 46, 47, 48, 49) and tyrosinosis (9).

The ingestion of other pure amino acids, including glycine, methionine and tryptophane, in comparable or even larger dosage failed to induce the appearance or raise the output of hydroxyphenyl derivatives.[9]

ERADICATION OF URINARY INTERMEDIATES

Role of Vitamin C.   Allusion has been made to the inconstant and irregular exhibition of the metabolic aberration by premature infants whose high protein diets had been fortified with vitamin C (10 or 20 mg. of l-ascorbic acid daily).   On such diets, roughly one-half, or 7 of 15 infants, excreted tyrosine derivatives in contrast to their consistent excretion in all 17 premature infants who had been on comparable but vitamin C–deficient diets for one week or longer.

Moreover, similar, small daily intakes of vitamin C frequently prevented the hydroxyphenyluria of both premature and full-term infants, produced by ingestion of the pure amino acids (9 of 15 observations).

This prophylactic role of vitamin C suggested that it might also possess curative properties in abolishing an already existent metabolic defect.   This supposition proved to be correct.   Typical examples are given in the following figures.

In every instance, the parenteral administration of l-ascorbic acid in dosages of 50 mg. or more abolished hydroxyphenylurias

[9] The rise in urinary amino acid nitrogen following ingestion of the synthetic amino acids, d,l-phenylalanine (previously mentioned) and d,l-methionine (and l-tryptophane) exceeded that following l-tyrosine and glycine, suggesting again the less economical utilization of the unnatural enantiomorph (4).

of high magnitude within 24 to 48 hours. The potency of small daily doses of 10 to 25 mg. by mouth was equally striking but somewhat delayed (J.C.).

The curative role of vitamin C is illustrated again in figure 9.

In one infant (R.M.G.), the parenteral administration of 50 mg. of ascorbic acid was ineffective but 100 mg. given three days later by the same route resulted in prompt cessation of hydroxyphenyluria. In both infants, the cycle of appearance of urinary
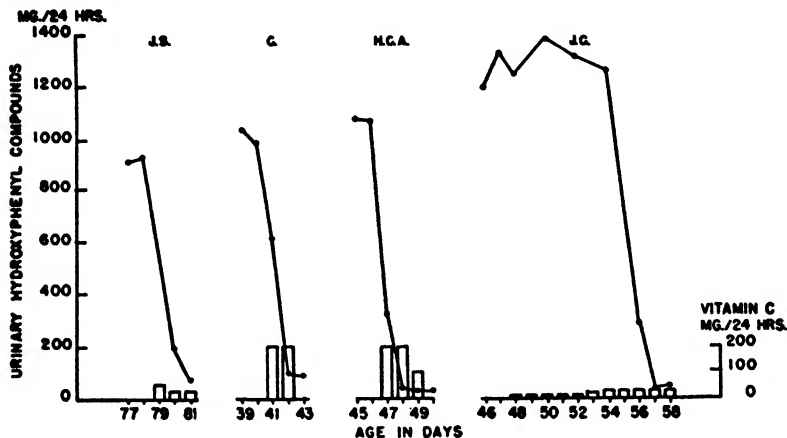

FIG. 8. Eradication of spontaneous hydroxyphenyluria in premature infants by vitamin C.

intermediates with omission of vitamin C and their subsidence with resumption of the vitamin was repeated on two occasions.

Vitamin C was equally effective in abolishing the hydroxyphenyluria precipitated in full-term infants by a single dose of the pure amino acids (figure 10).

L-ascorbic acid in a single dose of 200 mg. parenterally or 500 mg. by mouth caused a prompt subsidence of hydroxyphenyluria induced by phenylalanine ingestion.

The catalytic participation of vitamin C in intermediary protein metabolism probably explains the reported heightened requirements for this vitamin in guinea pigs receiving tyrosine (50), in premature and full-term infants receiving cow's milk

(51, 52, 53, 54) and the higher incidence of scurvy in artificially fed infants. The key position of this vitamin in the metabolism of phenylalanine and tyrosine in infants (3, 4, 5, 6), in animals (35, 55, 56) and *in vitro* (57, 58) is analogous to that reported for niacin in tryptophane metabolism (59, 60) and for pyridoxal phosphate in tyrosine decarboxylation in some strains of bacteria (61).
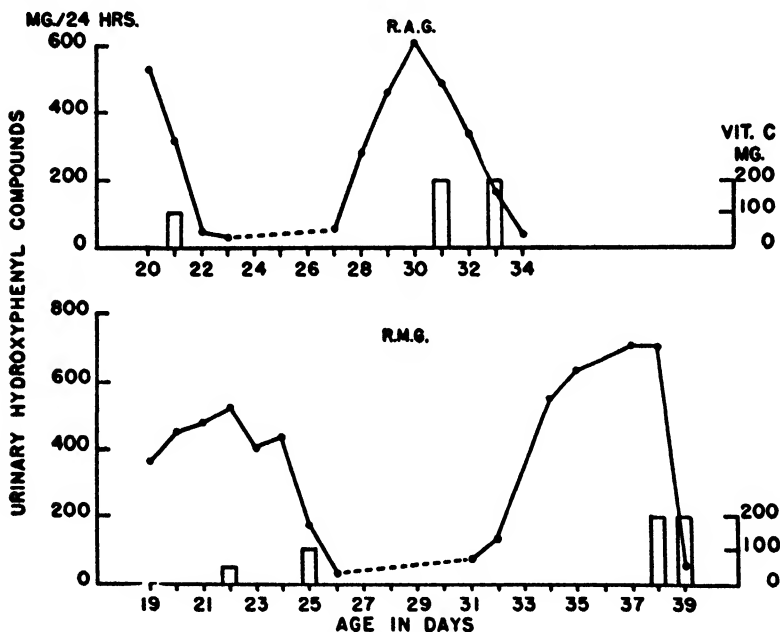

FIG. 9.   Recurrent hydroxyphenyluria and eradication by vitamin C.

The evidence thus far presented seems adequate to establish that exhibition of the metabolic aberration by infants is an interrelated function of three variables: aromatic amino acid intake, fetal maturity, and vitamin C content of the tissues. The quantitative nature of the interrelation of the first two factors has already been considered. Quantitative differences in the body stores of vitamin C in premature and full-term infants (62, 63, 64) suggest a similar interrelation between the last factor and
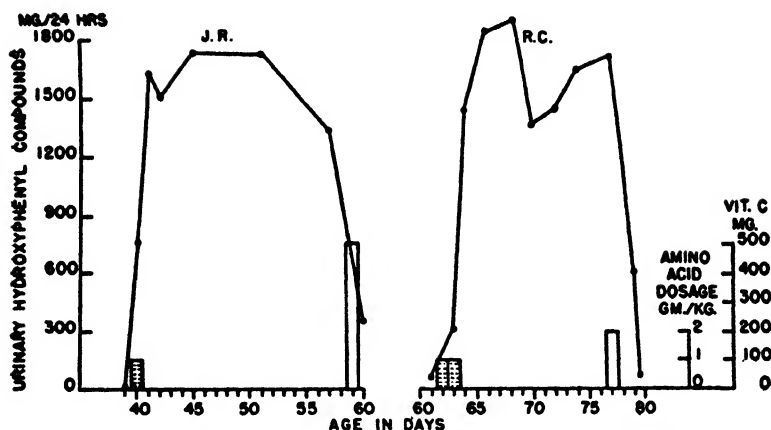
FIG. 10. Eradication of artificially induced hydroxyphenyluria in full-term infants by vitamin C.

fetal maturity. Evidence, now offered, likewise demonstrates a quantitative relation between vitamin C and aromatic amino acid intake.

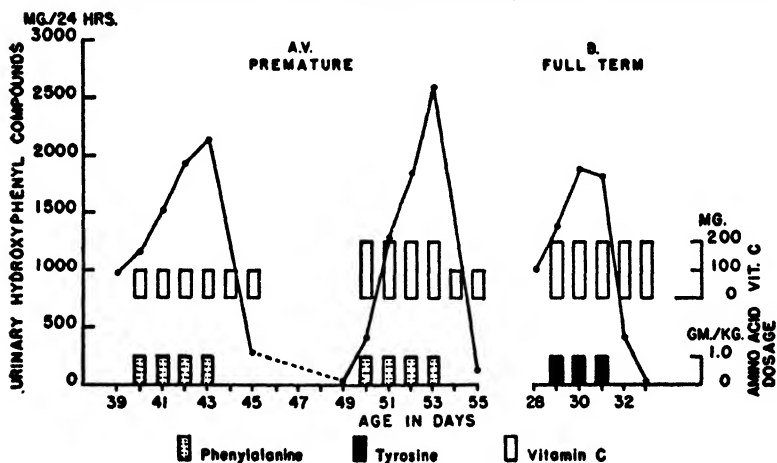When repeated and high dosages of the pure amino acids were

FIG. 11. Failure of vitamin C to eradicate hydroxyphenyluria induced by repeated dosage of amino acids.

given to both premature and full-term infants, the consistent effectiveness of vitamin C, hitherto observed, was no longer demonstrable (figure 11).

Despite the concurrent administration of l-ascorbic acid in large and repeated dosage, the excretion of hydroxyphenyl compounds (and phenyl compounds with phenylalanine ingestion, not shown in figure 11) frequently persisted at high levels throughout the periods of amino acid administration. Presumably, the large amounts of incoming amino acids overwhelmed the vitamin's opposing action by temporarily deranging the cellular enzymatic mechanisms responsible for aromatic amino acid degradation in the body. This concept is supported by the failure of vitamin C, irrespective of dosage, to influence the pattern of excretion during the acute phase of derangement. The magnitude of hydroxyphenyluria (and phenyluria) was frequently the same, whether the simultaneous intake of l-ascorbic acid was 25 mg. daily or as high as 400 mg. daily and presumed tissue saturation. The complete and prompt reversibility of the derangement was, however, proved by the immediate fall in urinary intermediates with stoppage of amino acid intake and continued vitamin C administration in the post-periods.

A study of scorbutic patients and normal children and adults (55) was not undertaken, but the quantitative nature of the metabolic error suggests that, under suitable conditions of aromatic amino acid satiety and tissue vitamin C depletion, even their enzymatic mechanisms might be overwhelmed to the point of exhibiting the defect.

Plasma Ascorbic Acid Levels. Frequent analysis of the blood plasma for ascorbic acid (65) in the vitamin C–deficient periods always gave values below 0.1 mg. per 100 cc. Of greater moment was the common observation that full correction of the metabolic defect by vitamin C was effectuated without appreciable elevations of plasma levels (0.3 mg.%, more often under 0.1 mg.%). Replenishment of intracellular stores sufficient to remedy tissue enzymatic deficiency antedated and was not reflected in a rise of circulating ascorbic acid. None of the infants showed clinical or roentgenographic signs of scurvy.

The absence of clinical scurvy with full exhibition of the metabolic error and the absence of changes in circulating ascorbic acid in the face of full repair of the defect in cellular metabolism are in accord with published data (66, 67, 68, 69, 70). The appearance of tyrosine intermediates in the urine of infants undoubtedly represents an extreme degree of vitamin C desaturation of the tissues; their eradication, when previously present,

TABLE 5

EFFECT OF OTHER AGENTS ON METABOLIC DEFECT

INEFFECTIVE

VITAMINS		OTHER AGENTS
A	Thiamin	Cortate
D	Riboflavin	Creatine
α-Tocopherol	Niacin	
B Complex	Pyridoxine	
Ryzamin, Yeast,	Biotin	
Crude liver extract	Choline	

PARTIALLY EFFECTIVE

Liver Extract E 795
d-Isoascorbic Acid

may constitute an early and sensitive indicator of repair of intracellular vitamin C deficiency. The ascorbic acid content of the white cell layer (71, 72) which is presumably a better index of tissue concentration than plasma levels, was not determined.

Role of Other Dietary Factors. The prophylactic and therapeutic efficacy of vitamin C is apparently specific since no other dietary substances consistently remedied the metabolic defect.

The agents listed in the table were given singly, in combination, and many of them in summation over prolonged periods and in

ample dosage by mouth and parenterally with negligible effect. The lack of potency of thiamin chloride in liberal dosage (20 mg. daily for long periods) differentiates this metabolic error from the phenylketonuria induced in vitamin B–deficient rats by phenylalanine ingestion (73). This relation, however, remains unconfirmed (74).

Liver extract, E–795, with added thiamin and riboflavin[10] (vitamin C-free) produced a prompt and persistent eradication of artificially induced hydroxyphenyluria in one full-term infant. It was, however, given with no or slight success to four premature infants and vitamin C–deficient guinea pigs.[11] If this difference in response between premature and full-term infants is confirmed, it would suggest that full fetal maturity may bring with it a factor or factors, perhaps of hepatic origin (16, 75, 76, 77) and not solely dependent on vitamin C, capable of assisting in the breakdown of aromatic amino acids.

The oral administration of 500 mg. of isomeric d-isoascorbic acid to one premature infant resulted in a transient decrease of approximately 20 per cent in urinary output of hydroxyphenyl compounds with a return to fore-period levels on the third day following therapy. The minimal effect of this agent is commensurate with its recognized lesser antiscorbutic potency (56, 78, 79, 80).

The potency of acids, other than l-ascorbic and d-isoascorbic, and the neutralizing effect of alkali, demonstrated in the experimental alcaptonurias of rats (73) and guinea pigs (81) were not tested in this study.

COMMENT

Physiologic Considerations. The intermediary metabolism of aromatic amino acids in the human organism is not established with certainty but the simplified diagram in the figure portrays the generally accepted pathways (82).

[10] Eli Lilly and Company.

[11] Unpublished experiments by Dr. M. Dann showed that the hydroxyphenyluria induced in vitamin C–deficient guinea pigs by tyrosine ingestion was not affected by this agent in liberal dosage (0.4 to 1 cc. by mouth or parenterally).

The amino acids, phenylalanine and tyrosine (p-hydroxyphenyl-alanine), are deaminized to their respective keto derivatives, phenylpyruvic acid (22, 25, 83, 84) and p-hydroxyphenylpyruvic acid (25, 84, 85, 86).   In the metabolism of phenylalanine, an hydroxyradical may irreversibly be added to the phenyl ring in the para position to form tyrosine so that the keto acid of tyrosine may be an intermediate of both amino acids (87).   The keto acids, by reversible reactions, are then reduced in part to phenyl-lactic and p-hydroxyphenyllactic acids and in part oxidized
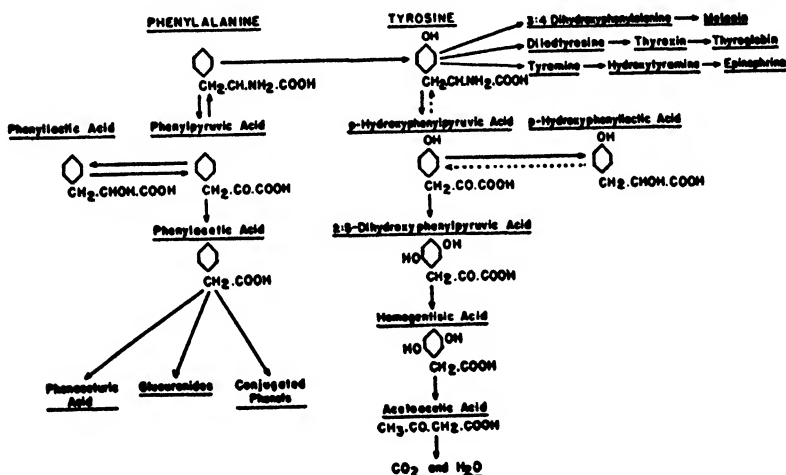

FIG. 12.   Intermediary metabolism of aromatic amino acids.

further to phenylacetic (and phenylproprionic) acid and homo-gentisic acid.   These acids are next degraded and conjugated to appear in the urine as phenaceturic acid (88, 89), glucuronides and conjugated phenols and cresols (86, 90), or they are com-pletely oxidized through an acetoacetic stage to carbon dioxide and water.

The described aberration of aromatic amino acid metabolism in infants differs in many respects from the known inborn errors of metabolism, phenylketonuria, alcaptonuria and tyrosinosis, and from the experimental hydroxyphenylurias of animals.

In phenylketonuria which is characterized by mental deficiency and the excretion of phenylpyruvic acid in the urine (73, 91, 63), the enzymatic defect is limited to an inability of the organism to form p-hydroxy analogues from phenylalanine and phenylpyruvic acid. The metabolism of tyrosine is unimpaired (27, 44, 92). In alcaptonuria, the defect resides in the specific inability to catabolize homogentisic acid which in consequence appears in the urine. This error also stems from the genetic absence of a specific enzyme which is found in the blood of normal persons (93, 94). In a single patient with tyrosinosis (9), the point of blockage was found to be at the stage of oxidation of p-hydroxyphenylpyruvic acid to its 2,5-dihydroxy analogue. Oxidation of ingested homogentisic acid was complete. Large amounts of the keto and lesser amounts of the oxy acid of tyrosine and of 3,4-dihydroxyphenylalanine were excreted in the urine. In albinism, the enzymatic block is at some point between tyrosine and melanin and the enzyme systems at fault are presumably tyrosinase or dopa-oxidase, or both. In none of these congenital errors of metabolism is vitamin C effective in therapy (27, 47, 95).

In the experimental alcaptonurias of animals induced by amino acid ingestion (17, 31, 56), homogentisic acid is usually reported as the major excretory product with lesser amounts of the keto and oxy acids of tyrosine. Only in scorbutic guinea pigs has the prophylactic and therapeutic potency of vitamin C been demonstrated (96). According to recent reports (97, 98), the point of blockage and the site of action of the vitamin in these animals appear to be at a stage prior to the formation of the keto acid of tyrosine, and l-phenylalanine, l-tyrosine, phenylpyruvic acid, and l-3,4-dihydroxyphenylalanine seem to be the only aromatic compounds dependent on vitamin C for their degradation.

The site of blockage of the metabolic aberration herein described resembles that in tyrosinosis but it differs from this inborn error in the larger excretion of the oxy than the keto acid of tyrosine and in its reversibility by vitamin C. It differs from phenylketonuria and the alcaptonurias of man and animals by

the transient excretion of phenylpyruvic acid even with phenyl-alanine ingestion and the invariable absence of homogentisic acid from the urine. Vitamin C deficiency does not preclude the continued *in vivo* formation of p-hydroxy analogues from phenyl compounds (the site of blockage in phenylketonuria). The ingested and converted amino acids are in part oxidized to keto acids, chiefly of tyrosine, and in larger part reduced further to oxy acids, again chiefly of tyrosine. These intermediates are eliminated in the urine without a concomitant rise of any of the lower degradation products. Only with ingestion of the pure amino acids, do the amino acids themselves leak into the urine in substantial amounts, presumably by simple overflow.

The cellular enzyme mechanisms at fault in this human aberration would seem to be related more to tyrosinase and decarboxylase than to amino acid deaminase function. The physiologic reduction in the general functions of the liver and kidney of the premature infant suggests by analogy a similar impairment of enzymatic function since these organs are known to be prominent sites of origin of the first two enzymes (39, 99). The excretion of deaminated organic acids containing the intact phenol ring further points to a failure of decarboxylation (decarboxylases) and not of phenol oxidase function as the primary mechanism at fault. It has recently been shown that liver slices of vitamin C–deficient animals can metabolize the aromatic ring of tyrosine, as indicated by the disappearance of hydroxy groups (39) whereas they and kidney slices (to a lesser degree) are unable to oxidize the side chain of tyrosine (100). In the latter study, repair of the defect could be effected *in vivo* and *in vitro* by vitamin C. If the faulty mechanism is the same in infants as in these animals, the curative properties of vitamin C may reside in its ability to catalyze tyrosine decarboxylation in a manner analogous to that of pyridoxal phosphate in certain strains of streptococci (61).

Clinical Considerations. The clinical significance of the metabolic defect is still conjectural. Even those infants who exhibited the defect over prolonged periods continued to thrive and showed

no overt signs of either amino acid or vitamin C deficiency. They gained weight well, were in positive nitrogen balance and signs of scurvy were absent, both clinically and by roentgenogram. Despite the negative evidence, the observations give rise to a number of intriguing clinical questions. What is the relation, if any, between the demonstrated defect in the metabolism of tyrosine and phenylalanine and the morphologic changes characteristic of vitamin C deficiency, namely, improper formation of intercellular substance and collagen (101, 102)? Since tyrosine is a precursor of both thyroxine and adrenalin, may the presence of the defect in premature infants contribute, under certain conditions, to their instability of body temperature regulation? Is the delicate transparency of the skin of premature infants related to improper utilization of melanin, another product of tyrosine? Does the observed increased excretion of aromatic organic acids in premature infants fed cow's milk play a role in their known tendency to develop severe rickets? If so, this observation may explain, in part at least, the frequently postulated importance of vitamin C in the development of rickets. From the practical standpoint and despite the absence of overt signs of deficiency, the reported observations emphasize the importance of early and ample administration of vitamin C to all premature infants receiving cow's milk mixtures of high protein content.

As with most investigations, the questions answered in the course of this study have raised more questions which still await solution.

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